Western Reserve University
Cleveland

# Collected Papers
from
# The H. K. Cushing Laboratory of Experimental Medicine

EDITED BY
G. N. STEWART, M. D., DIRECTOR

Volume II
1912-1913

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# EXPERIMENTAL STUDIES OF PARALYSES IN DOGS AFTER MECHANICAL LESIONS IN THEIR SPINAL CORDS WITH A NOTE ON 'FUSION' ATTEMPTED IN THE CAUDA EQUINAS OR THE SCIATIC NERVES

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TWENTY-SEVEN FIGURES

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## INTRODUCTION

### *Reasons for the research*

The present series of experiments was begun in the spring of 1908 with the production of mechanical lesions in the lumbar spinal cords of dogs. The object was to bring about distinct motor paralyses. After the animals had reached permanent stages in the paralysis, so that there was no question about degeneration in the roots and peripheral nerves, it was planned to attempt to distort the nerve-patterns in these structures, using for this purpose a method heretofore apparently untried. This method is referred to as nerve 'fusion,' and consists simply in uniting two or more nerves by tying them together with absorbable ligatures.

In carrying out the plan given, it early became apparent that the subject of nerve 'fusion' was a research in itself, requiring many special experiments. Fusions were therefore attempted on normal nerves where no central lesions had been produced. A preliminary report of this work has already appeared[1], and we have been able to offer some evidence that after nerves are united by this method, a certain amount of distortion of pattern may take place.

However, even if changes in nerve-pattern are obtainable by the union of nerves which are normal, it does not follow that the same changes may be expected where there has been a central lesion. Fatty degeneration takes place in the paralyzed muscles and in the nerve trunks themselves, the degenerated tracts close up and become replaced by new connective tissue.

Therefore, the original design of producing central lesions and later trying nerve fusions has been carried out. In the course of this research two other issues have developed which proved to be of equal if not greater interest. It was shown that after a lesion was produced in the spinal cord by the method which will be outlined, a very characteristic spontaneous recovery took place similar to the early recovery often seen in infantile paralysis, and it was found that the material furnished data for adding to the precision of our knowledge on the localization in the lumbo-sacral cord certain nuclei of the pelvic viscera and of the muscles of the hind limb and tail.

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[1] Feiss: Boston Medical and Surgical Journ., May 11, 1911.

This report may therefore, be divided into three parts; in the first part attempting to account for the clinical improvement occurring after the lesion, in the second part showing the relation of the autopsy findings to the peripheral and visceral palsies produced, and in the third part discussing the subject of fusion and giving a brief account of some experiments in which, some months after the lesion, this procedure was attempted.

### *The production of the lesion*

The lesions were made subdurally through a single trephine opening, usually at the 4th or 5th lumbar spine. The instrument for the purpose (fig. 1) was L-shaped, the longer arm forming the handle and the shorter shaped into a thin blade. This was entered through the dura in the median line and then moved laterally with sufficient force to crush the cord substance. Both unilateral and bilateral lesions were attempted. The operations were done under complete ether anesthesia with the usual precautions for asepsis. All told, lesions were attempted in some seventy-five animals, but of course, on account of various causes, including an epidemic of distemper, the majority of animals were lost.

### *Method of studying the surviving animals*

Observations were made from day to day and notes were made on the changes. At intervals of 2 or 3 months, each dog was studied more systematically, according to the following routine:

1. Attitude
2. Gait. Nature of limp if present
3. Active movements: rump, hips, knees, ankles, paws and tail
4. Passive movements: rump, hips, knees, ankles and paws
5. Response to stimulation; sharp point applied to skin of various parts of limb
6. Response of paw to heat with immersion in hot water (heat-pain sense)
7. Temperature of skin between toes

8. Measurements. Circumferences above paw, ankle and knee
9. Reflexes: knee jerks; anal reflex (observed by inserting glass rod into anus)
10. Control of sphincters (based on ordinary clinical observations)
11. Response to faradism; applied to various parts of limb
12. Remarks
13. Photograph

In a few of the dogs data bearing on spinal localization could be obtained by direct stimulation of peripheral nerves, which was done at the time of the secondary operations of nerve fusion.

### *Study of autopsy material*

The spinal cords were studied in twenty of the cases. In six of them proper identification of the roots could not be made, owing to scar. The other fourteen were each mounted on cardboard, and the roots spread out, stitched down, and properly numbered. The count was made from the thirteenth, using the last rib as a land-mark. Each mounted specimen was sketched and placed in Müller's fluid.

The boundaries of the cord segments were usually estimated by means of their relations to dural root exits, this relationship having been based on a number of dissections of normal cords.

After proper hardening, the cord was cut transversely into a number of pieces without attempting to remove the dura, so as not to disturb the rootlets within. The exact location of each of these cuts in relation to dural root exits was indicated on the sketch. The pieces were cut 2 to 3 mm. thick and imbedded in celloidin. Sections from each piece were stained by at least three methods, Hematoxylin-eosin, Van Gieson and Weigert-Pal.

## PART 1. CLINICAL IMPROVEMENT AFTER THE PRODUCTION OF THE LESION IN RELATION TO THE HISTOLOGICAL FINDINGS AT DIFFERENT STAGES

This portion of the research is based on the studies of twenty dogs and their spinal cords. The important points are summarized in table 1. Glancing at this, it is seen that after such a lesion as described, almost all the animals showed more or less clinical improvement. It is further seen that this improvement fell into two stages, (1) a sudden improvement occurring in the first or second week, and (2) a slow improvement continuing for three or four months.

### *Character of the clinical improvement*

The paralysis immediately following the operation was often a complete paraplegia, sometimes of one leg only, and rarely of the tail and sphincters. The dog lay in the kennel, seemingly stunned and not caring for food. He appeared feverish and thirsty. After a day or two, in spite of the paralysis, he made some attempt to get over the ground, with but partial success. The sudden improvement in the first or second week was often very marked, a paraplegia sometimes changing into a mere turning of one paw. Figs. 2, 3, 4 and 5 show characteristic changes in two of the dogs. Whether or not the improvement began suddenly, there was always a period of gradual recovery ending in a permanent state of residual paralysis.

The question is, how much of the improvement is based on changes which are histologically demonstrable? The table (table 1) indicates what the chief histological changes were (the topography of ten of the lesions is studied in Part 2).

### *Histological summary*

In the early autopsies the location of the lesion was evidenced by a mass of débris, including broken down myelin in drops, leucocytes and round cells in clumps. A mass of granulation tissue had formed along the dura, at the place of injury. This entered the mass of débris. In this stage, cavities were not apparent, but in the neighboring grey matter were small areas of beginning necrosis.

In animals autopsied at the end of one month, the most noteworthy thing was the appearance of cavity-like spaces. These were formed of connective tissue bands some of which had become well defined owing to the partial removal of the tissue débris. By this time the scar connected with the dura had become quite dense.

At the end of two to three months, the sections showed the permanent histological characteristics of the lesion. These were either cavity spaces or scar, or both. The cavity spaces were sometimes sharply circumscribed, and occasionally their outlines corresponded to the original outlines of the grey matter. In general, it seemed that the amount of scar depended on the amount of direct injury to the pia and dura, and the size of the cavity-spaces on the extent of the original destruction of cord substance, although of course, the extent of the original cavities could not be completely judged on account of tissue repair. In some places the grey matter showed marked distortion of outline, suggesting contraction.

As to signs of nerve regeneration in the cord, one occasionally saw among the débris and granulation tissue small fibers with faint coats of myelin. But of course, there was no way of telling whether these indicated repair; if they did it could have been slight only.

In all stáges, one saw striking changes in the ganglion cells of the gray matter, near the lesion. These consisted in alterations in size, shape and staining reactions as well as chromatolysis and vacuolation.

### *Inferences from the above*

Judging from the histological condition one could divide the anatomical appearances into two stages, roughly corresponding to the two stages in the clinical improvement. It seemed likely that the early improvement was partially due to the removal of tissue débris, because the cavities became apparent about that time. Also as shown clinically, by the swelling about the wound, there was at first marked fluid exudate, the absorption or escape of which was undoubtedly a factor in that improvement.

As to the more gradual improvement that supervened in most of the cases, disregarding the anatomic evidence of repair of nerve tissue in the cord itself, as being too slight to explain the marked restoration of function, there are four other explanations to be considered: (1), actual regeneration of nerve fibers in roots and peripheral nerves; (2), vicarious activity of nerves and muscles (including employment of other paths in the cord); (3), failure of many of the neurones originally pressed on by the exudate to degenerate completely, so that with removal of the exudate they recovered their functions; (4), changes in subjective state in the animal as brought about by lessened discomfort. (The last two explanations might also apply to the early improvement.) We cannot say which of these factors were most important—in fact, it is possible that all of them had a share in explaining the clinical change for the better.

### *Previous researches on the subject*

That marked restoration of power may occur after lesions in the central nervous system is well known. This applies both to cortical extirpations,[2] and to hemisections in the cord.[3] The fact that there is also considerable restoration of power after complete section would seem to indicate that there might be regeneration across the scar. This has been actually observed in some of the lower animals.[4] Brown-Sequard noted it in fishes and Fraisse in amphibians. But in higher animals it has not been observed,[5] and for this reason, in cases of hemisection restoration of the clinical state has been said to be partially due to the employment of paths on the contralateral side. Be that as it may, it is not necessary to base the restoration of function on nerve repair across the scar because the clinical improvement seemed practically as great in our cases where, on account of the extent of the lesion, conditions for nerve repair were not favorable and where, moreover, signs of such repair were scarcely to be made out.

### *Bearing of above inferences on infantile paralysis*

The spontaneous restoration of power noted in our dogs is very similar to that often observed in infantile paralysis. This applies both to the early and late improvement. The former, also in that disease, is at least partially due to the removal of inflammatory material, somewhat as in the conditions described above, where the exudate is dependent only on simple mechanical lesions, although part of the recovery in infantile paralysis may be related to cell changes which take place as a result of the expulsion or neutralization of specific toxines. As regards the later improvement in that disease, one is just as much in doubt as to which of the factors above mentioned is of greatest significance.

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[2] Sherrington: Integrative action of the nervous system, 1906, p. 277.
[3] Weiss: Sitz. d. Akad. d. k., Wissensch., Wien, 1879, Bd. 80.
[4] Bechterew: Functionen der Nerven Centra, 1908, vol. 1, p. 652.
[5] Schiefferdecker: Virchow's Archiv, Bd. 67.

It is plain, however, that the spontaneous recovery observed in infantile paralysis is perhaps an attribute less characteristic of the disease than of the anatomical structures which the disease attacks. Whether it is a clean section, or a crushing lesion or an inflammation set up by some virus, there are certain succeeding manifestations that seem to be common to all these antecedents, and these manifestations are exhibited with striking clearness simply because of the fact that in the central nervous system, a relatively small anatomical change affects an extremely large physiological sphere.

## PART 2. THE LOCALIZATION OF CENTERS IN THE LUMBO-SACRAL CORD

### *Method of study and reasoning*

This part of the research is based on ten of the experiments. The study of data furnished by this material is summarized in tabular form (table 2) and each experiment is illustrated with a photograph, and a diagram based on the sketch made at the autopsy. In these diagrams only direct involvements due to the lesions are indicated. The degenerations were partly due to secondary operations, and are therefore best omitted, because under the circumstances they throw no light on the localization.

In handling the data, each case is analyzed for itself and the inferences individually derived are collated according to the important clinical and physiological headings under which each was studied. The cord segments were numbered according to the roots, calling the root issuing beneath the last rib, the 13th.

Unfortunately, the ribs themselves were not counted, so that the possibility of variation must not be lost sight of, as a source of error.

The most pertinent information is that obtained in the individual case by comparing rights and lefts. Other things being equal, one may in certain cases, ascribe a unilateral effect to a corresponding unilateral lesion. In the same way by proper elimination, bilateral effects may sometimes be ascribed to bilateral lesions. It will be apparent that the collation of all the evidence according to numerical segments offers less exact conclusions than those which can be derived in the same individual and more especially with reference to the relative position of centers. But by comparing relationships and detecting correspondence, certain inferences of significance may be obtained. Aside from the assumption that the upper leg centers are, generally speaking, higher than those of the lower leg, no further assumptions were used, except, of course, such as are based on the classical conceptions of the functions of anterior and posterior roots and of the white and grey matter of the spinal cord.

## *Inferences from individual experiments: summary*

*Experiment 64.* (Figs. 6 and 7.) External popliteal paralysis on left not accounted for by slight anterior root damage as there was similar damage on right where there was no paralysis. Therefore the external popliteal centers must be in the upper 7th lumbar segment, and by comparing right and left anterior horns, they must be in the dorsal two-thirds of the horn. Loss of left knee jerk accounted for by involvement of 5th and 6th posterior roots. If internal popliteal centers are at the level of or below the external popliteal centers (see Experiments 78 and 86), paths for voluntary control seem to run in the anterior column.

*Experiment 78.* (Figs. 8 and 9.) Weakness of right knee and hip best explained by involvement in 5th lumbar segment and anterior root filaments attached. As lesion in grey matter of middle 6th lumbar leg is bilateral and left leg seemed good, it could not account for paralysis of right external popliteal, which is consequently traceable to damage of right anterior roots attached to lower 6th and upper 7th lumbar segments. Both internal popliteals being good, their centers must be lower than external popliteal centers, for they could not be higher on account of bilateral lesion just above, which lesion also suggests a possible gap between upper and lower leg centers. Extinction of right knee jerk explained by 5th and 6th anterior root involvement. Voluntary dorsal and plantar flexion of left paw and ankle, and plantar flexion of right seems possible with only anterior columns open.

*Experiment 86.* (Figs. 10 and 11.) External popliteal paralysis on both sides due to damage in middle 6th and lumbar segments and roots attached. Almost complete paralysis of internal popliteal on right and not on left corresponds to lesion in upper 7th lumbar segment. Loss of point and heat-pain sensation on right and not on left explained by lesion in right half of cord in 5th lumbar segment. The difference in damage to grey matter in lower 6th and upper 7th lumbar segments must account for preservation of left knee jerk. As internal popliteal is good on left and its centers are in upper 7th lumbar segment, impulses from the brain to its synapses must have passed through the marginal portion of the anterior column in the segment above.

*Experiment 69.* (Figs. 12 and 13.) The difference between the right and left anterior horn involvement in the upper 7th might explain the weakness of the right external popliteal. Lesion in 6th lumbar segment suggests a gap between upper and lower leg centers. This lesion seems to have had no effect on either knee jerk or on sensations, suggesting that these latter must have entered through upper 6th rootlets or higher. As the centers presiding over control of right paw may be presumed to be below the 5th lumbar segment, the extent of the lesion might denote that such control was cut off on that side. Therefore, as the dog used the paw quite well (except for the external popliteal weakness mentioned) it is possible that impulses crossed from the other side, where the lateral column was good.

*Experiment 65.* (Figs. 14 and 15.) The extensive paralysis of the left leg and paw together with loss of knee jerk, best accounted for by severe root involvement, while the weakness in the right upper leg is accounted for by damage to anterior horn in lower 6th lumbar segment and some of the 6th anterior root filaments.

*Experiment 75.* (Figs. 16 and 17.) Here permanent flexure of right hip and stiffness of knee, perhaps due to weakness of gluteals and hamstrings respectively, caused by damage of 5th, 6th and 7th anterior roots. The lower leg paralysis explained by lesion in 7th lumbar and 1st sacral segments. Loss of right knee jerk explained by damage to 6th posterior root.

*Experiment 70.* (Figs. 18 and 19.) The lesion in lower part of 5th lumbar segment involved entire cord, practically acting as a trans-section. This accounts for loss of control and spasticity of hind parts, and places most of leg centers below that segment. It is likely that preservation of knee jerk, anal reflex and control of sphincters is due to sparing of centers below middle 6th lumbar segment (the anterior roots being also destroyed above that level).

*Experiment 72.* (Figs. 20 and 21.) The lesion in upper 5th, 6th and 7th lumbar segments, with the corresponding anterior root damage accounts for loss of control of hind parts, including point sensation, knee jerks and sphincters.

*Experiment 68.* (Figs. 22 and 23.) Impairment of control of motion, point and heat-pain sensation of tail, together with impairment of anal reflex and sphincteric control, all accounted for by lesion in 7th lumbar and 1st sacral segments. Slight paralysis of right paw also explained by damage to right anterior roots.

*Experiment 73.* (Figs. 24 and 25.) Impairment of control of motion, point and heat-pain sensation in tail, together with impairment of anal reflex and bladder control, all accounted for by lesion in the 7th lumbar segment.

### *Correlation of data derived from individual experiments*

*A. Control of joints.* Upper leg centers are mostly in the 5th and 6th lumbar segments, that is, about the level of the 4th dural root exit. Lower leg centers are in the lower 6th and the 7th lumbar segments, that is, at the level of the 5th dural root exits. Nuclei of the external popliteal, the internal popliteal and the tail are somewhat circumscribed and relatively isolated, and the nucleus of the external popliteal is, as a whole, higher than that of the internal popliteal. There may be some overlapping of nuclei, but in one portion of the cord, namely, the middle 6th lumbar segment, there seem to be relatively few centers, the nuclei of the upper and lower leg seeming to be respectively above and below this apparent gap. (Possibly the hamstring centers lie here.)

It is suggested that the anterior columns contain fibers from the brain which convey volitional impulses.

*B. Knee jerks.* Its extinction or weakening is consistent with corresponding damage to either the roots or grey matter of the 5th and 6th lumbar segments.

*C. Sensation.* As to point sensation of the whole leg, the 4th and 5th posterior roots seem important links in the afferent chain.

As to heat-pain sensation of the paw (studied by immersion in hot water), the test was omitted in three of the experiments and was always accompanied by loss of point sensation. In one case however (Experiment 70), point sensation was lost without corresponding impairment of heat-pain sensation in paw.

*D. Anal reflex.* This test was omitted in one case, found impaired in seven and normal in two.

*E. Control of sphincters.* Their centers lie below the 6th lumbar segment. It is suggested by comparison of two cases

(Experiments 68 and 73) that the bladder centers are higher than the anal centers. Sphincteric centers are very close to tail centers (5th dural exit).

*F. Conclusion.* Inferences cannot be drawn for every point investigated, but there is significance to certain isolated facts, such for example, as pertains to the relative isolation of the external popliteal and internal popliteal centers, suggesting perhaps that these nerves, which on account of their individual spheres of distribution control the best coördinated joint movements, have their nuclei relatively best defined. The main suggestion is that the grouping of cells seems to correspond at least roughly to the gathering of fibers in individual peripheral nerves.

### *Comparison of above inferences with current views on the subject*

As regards the cases where preservation of voluntary control in certain muscles seemed to depend on paths in the anterior columns, which alone were open, other experiments are on record,[6] which show that in dogs and other animals, these columns do convey motor fibers from the brain. There is also the possibility of the employment of paths on the contralateral side (cf. Part 1) and in one of our cases (Experiment 69) this seems to be the only explanation for preservation of control of the paw, for the lesion had blocked all the homolateral paths higher up.

With reference to the knee jerk our localization in the 5th and 6th lumbar segments corresponds to Sherrington's more accurate findings.[7]

As to sensations, anal reflex and relative warmth of the paws, our findings are too few to be entitled to colligation with those of others.

The most important question that we have to consider is the significance of the cell-groups in the grey matter of the cord. Three methods have been previously used to investigate this point, the first being that of direct stimulation of spinal roots. The findings depend on the presumption that the cells of origin of the fibers in a given root are about on a level with the superficial origin of that root from the cord. Sherrington[8] has advanced some evidence for this, which evidence is based partly on the fact that after section through the cord just above a given anterior root, very little degeneration is to be seen in the fibers of that root, and partly on the results of direct stimulation of roots above and below the place of section. Besides Sherrington's contribution important reports on the results of direct stimulation of roots have been made by Bikeles and Gizelt,[9] Langley,[10] and Risien Russel.[11] The subject has been studied in connection with the formation of the lumbo-sacral plexus. The results agree fairly well, the fibers constituting the main nerve trunks being said to arise from the cord in the following descending order: crural, obturator, gluteal, sciatic, tail and sphincters. The internal popliteal fibers are, as a whole, placed higher than the external popliteal. There is supposed to be considerable overlapping of nuclei. Further than this longitudinal relationship, little information is obtainable by the method. Our results conform fairly well to the above order except as regards to relative height of the external popliteal and internal popliteal.

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[6] Bechterew: Functionen der Nerven Centra, 1908, vol. 2, p. 667.

[7] Sherrington: Schaefer's Text-book of Physiology, 1900, vol. 2, p. 874.

A second method is based upon the pathological findings in human beings in such conditions where definite motor paralysis were clinically under observation[12] (infantile paralysis, tumors of the cord, etc.). The observations are, however, very fragmentary.

The third method consists either in the amputation of limbs or parts of limbs, or in the excision of peripheral nerves, and later studying the ganglion-cell changes to be observed in the spinal cord. Valuable contributions have been those by Sano,[13] Van Gehuchten and Nelis,[14] Flatau,[15] Marinesco,[16] Bruce,[17] and

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[8] Sherrington: Journ. of Physiol., vol. 13, 1892, p. 621.

[9] Bikeles and Gizelt: Pflüger's Archiv, 1905, vol. 106, p. 43.

[10] Langley: Journ. of Physiol., 1891, vol. 12, p. 347.

[11] Risien Russel: Proceed. Royal Soc., 1894, vol. 54, p. 243.

[12] Wiekmann: Die Rückenmark-nerven und ihre Segment-bezüge, Berlin, 1901.

[13] Sano: Les localizations des functions motrices de la moelle épinière, 1908.

[14] Van Gehuchten and Nelis: Jour. de. Neurol., 1898, p. 301.

[15] Flatau: Archiv f. Anat. u. Physiol. Physiol. Abt., 1898, p. 112.

[16] Marinesco: Revue Neurol., 1898, p. 483.

[17] Bruce: Topographical atlas of spinal cord. 1901.

Knape.[18] Knape is practically the only one of these who leans toward the theory that cell groups represent collections of fibers in peripheral nerves. Even he does not describe sharply circumscribed nuclei as representing these nerves. He let his animals run a long time after excising the nerves, and before he studied the cords, in several cases allowing an interval of almost five years to intervene. His nuclei extend over more segments than our own.

As to previous observations on sphincteric control, reliance has usually been placed upon the stimulation of roots.[19] According to most observers[20, 21, 22] the nerve supply affecting control of micturition in dog and cat comes from two sources in the cord, an upper from the 3d, 4th and 5th lumbar roots, and a lower from the 2d and 3d sacral roots. Nerve fibers are sorted out in the hypogastric plexus before they finally pass to the bladder itself. According to Bechterew,[23] Sherrington,[24] Langley and Anderson,[25] and others, the lower source is especially important. This does not conform to our localization for bladder control in the 7th lumbar or 1st sacral segments.

Very much like the bladder, the rectum receives its nerve-supply from two sources,[26] and from about the same spinal nerves. Moreover, as in the case of that organ, the sacral nerves are much more important than the lumbar. Masius,[27] and Ott,[28] like ourselves, place the center higher than the lower source given by the others.

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[18] Knape: Ziegler's Beiträge. 1901, vol. 29, p. 251.

[19] Langendorff: Nagels Handbuch der Physiologie, vol. 4, 1st half, p. 350.

[20] Nawrocki and Scabitschewsky: Pflüger's Archiv, 1891, vol. 48, p. 335, vol. 49, p. 141.

[21] Budge: Zeitsch. f. rational med., 1864, vol. 21, pp. 1 and 174.

[22] C. C. Stewart: Amer. Jour. of Physiol., 1899, vol. 2, p. 182.

[23] Bechterew: Functionen der Nerven Centra, 1908, vol. 1, p. 292.

[24] Sherrington: Schaefer's Text-book of Physiology, 1900, vol. 2, p. 874.

[25] Langley and Anderson: Jour. of Physiol., 1895, vol. 19, p. 71.

[26] Starling: Schaefer's Text-book of Physiology, 1900, vol. 2, p. 336.

[27] Masius: Bull. Acad. Royal de Belgique, pp. 67, 68.

[28] Ott: Jour. of Physiol., 1879, vol. 2, p. 54.

### *The significance of cell-groups in the grey matter of the spinal cord*

According to different authors, the cell-groups in the gray matter of the cord are variously supposed to represent muscles, peripheral nerves, primary metameres or movements (as in the cortex). After a rather careful scrutiny of some of the literature, beside that mentioned above, we feel that in the present state of our knowledge, there is not sufficient evidence for any of these explanations. It is by no means certain that these cell-groups have any physiologic significance whatever. According to Knape, as above shown, and according to our own experiments, the findings seemed to point somewhat toward the peripheral nerve theory. Another piece of evidence for this theory is the fact that some of the cranial nerves have their cells of origin grouped in fairly well circumscribed nuclei. But neither is this analogy, nor the other evidence which we have cited, of sufficient weight to carry conviction. If the grouping of cells in the cord is ever susceptible of explanation, much further investigation will be required.

## PART 3. NERVE FUSION ATTEMPTED IN THE CAUDAS AND THE SCIATIC NERVES IN ANIMALS PARALYZED BY MECHANICAL LESIONS IN THE SPINAL CORD

This part of the research is based on eight cases, the only ones in which the animals survived both the original spinal lesion and a secondary 'fusion' done some time after they had reached permanent states in their paralyses.

### *The aim of the procedure*

Nerve fusion, it has been stated, consists simply in uniting two or more nerves by tying them together with absorbable ligatures. In the preliminary report[29] the theoretical basis for this procedure is given, the important point being that the direction of fibers regenerating in scar is governed by conditions offered by the mass of proliferated cells and nuclei which are here formed. As these are laid down in all directions the new fibers which develop in the interstices of the cells must grow accordingly. Therefore it

[29] Feiss: Boston Medical and Surgical Journ., May 11, 1911.

may be hoped that permanent changes in nerve pattern might result, and if two or more nerves are joined, that the mechanical attributes of the scar might cause fibers from fascicles of one nerve to pass into those of another. Besides, as shown by Perroncito,[30] Bethe[31] and others, one might even hope for branching of some of the regenerating fibers, so that if certain tracts, previously emptied by the paralysis, are entered by the new branches, there might result not only a change in nerve pattern but perhaps also a relative increase in the number of fibers. The purpose of the ligature is thus seen—it not only brings the nerves into physical apposition, but it also crushes them so as to cause the scar to form. Being absorbable (cat-gut) it disappears of itself. Compared with the older method of nerve crossing by suture, the theoretical advantages are: (1), that no division of nerves may be necessary; (2), that as many nerves as are in physical proximity may be included in the fusion; and (3), that change of nerve pattern may be hoped for in the individual nerve, even if no other unites with it.

Of special interest is the fact that in the cauda, at the region of the interspace between the dural exits of the 5th and 6th lumbar roots (dog) one may intradurally gather all the roots which supply the hind limb and tail into a compact bundle, and fuse them in the manner suggested above. In fact, by retracting the sensory roots, the motor roots alone may be thus joined together.

Below are given summaries of experiments in which fusions were attempted either in the cauda or in the sciatic nerves, some months after primary lesions were produced. These lesions have already been described (Part 2). The essential facts in the secondary fusions are given in table 3.

### *Experimental data*[32]

*Experiment 64.* Lesion, January 18, 1910. On April 27, 1910, residual paralysis (fig. 6) chiefly of left external popliteal. On this date, following operation, left sciatic exposed, and after faradic stimulation[33] determining that there was no response in the external popliteal the two popliteals (sciatic) crushed with haemostat and tied together with three cat-gut ligatures one-quarter inch apart. On August 19 (114 days after fusion), same nerves exposed, and firm neuroma found at place of fusion. Faradic stimulation of external popliteal showed flexion of toes, but no extension. Internal popliteal responded normally. On November 6 (193 days after fusion) similar responses and animal sacrificed. No clinical improvement noted.

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[30] Perroncito: Ziegler's Beiträge, 1907, vol. 42, p. 354.

[31] Bethe: Pflüger's Archiv, 1907, vol. 116, p. 385.

[32] All exposures of roots and nerves were made under full ether anaesthesia.

[33] In all the experiments a du Bois coil with 10,000 windings of the secondary and a two-pint Daniell cell in the primary current, were used.

TABLE 3

| NUMBER OF EXPERIMENT | DISTRIBUTION OF PARALYSIS | FUSION | | RESULTS OF FUSION | | |
|---|---|---|---|---|---|---|
| | | Number of days after date of lesion | Kind of fusion | Clinical (functional) | Physiologic (faradic) | Anatomic (myelination) |
| 64 | Paw and ankle E. P. | 97 | Popliteals | Slight (?) improvement | Negative | Some regeneration in and below neuroma |
| 69 | Paw and ankle E. P. | 123 | Popliteals | Worse than before | Negative—Stumps not united | Some regeneration in peripheral stump |
| 78 | Paw and ankle E. P. | 129 | Popliteals | No improvement (death in 60 days) | No tests | Beginning regeneration in neuroma. None in E. P. below neuroma. |
| 65 | Most of leg | 99 | Ant. roots in cauda | As before | Doubtful | Slight regeneration |
| 70 | Most of leg | 124 | Ant. roots in cauda | Worse than before | Responses in roots and nerves | Fair regeneration below neuroma |
| 75 | Most of leg | 122 | Ant. roots in cauda | Worse than before | No tests | Slight regeneration below neuroma |
| 68 | Tail | 85 | Cauda-Sacral and coccygeal roots | Fair improvement | Good responses | Some regeneration in and below neuroma |
| 73 | Tail | 111 | Cauda-Sacral and coccygeal roots | Fair improvement | Fair responses | Some regeneration in and below neuroma |

Anatomic report (In this and the following experiments, sections were stained by Weigert-Pal and general methods): Left sciatic above scar showed normal fibers and some unusual patches of connective tissue. Sections through midst of neuroma showed many small, and partially myelinated fibers some in bundles and others scattered among the cells of the scar. Scar dense and contains numerous nuclei. Popliteals below scar show scattered fibers with large interspaces and myelination, although not complete further advanced than in scar. Some large areas where no fibers appear suggesting that sheaths emptied by lesion, have not been filled.

*Experiment 65.* Lesion, January 19, 1910. On April 28, 1910, residual paralysis (fig. 14) chiefly of left leg. On this date, following operation: 6th and 7th arches removed, dura opened, cauda exposed. Sensory roots lifted aside and such motor roots as lay in the field stimulated. No response except in tail. Motor roots fused with one cat-gut ligature and crushed with haemostat. Dura sewed. Wound closed tight. No clinical improvement noted after operation. On September 26, 1910, stimulation of roots attempted with doubtful results. Animal sacrificed.

Anatomic report: Partial disappearance of anterior roots at region of fusion. Some scar with only a few nerve fibers interwoven in it. Roots peripheral to scar show greater numbers of fibers and myelination further advanced.

*Experiment 68.* Lesion, March 17, 1910. On June 10, 1910, residual paralysis (fig. 22) chiefly of tail and slight weakness of dorsal flexion of right paw. On this date following operation: 6th lumbar arch removed and all sacral and coccygeal roots (both anterior and posterior) constituting cauda at this region, fused with two cat-gut ligatures. No change noted after operation till in September or October, when tail seemed to be moved better (figs. 26 and 27). Thereafter but little gain. On November 30, 1910 (173 days after fusion), all roots divided and stimulated peripheral to fusion-neuroma and found to evoke tail movements. Animal sacrificed.

Anatomic report: Place of fusion shows dense and knotty scar with large numbers of cells running in all directions. Among these, small and partially myelinated nerve fibers have formed. Roots peripheral to scar contain fibers better myelinated, some almost normal.

*Experiment 69.* Lesion, March 17, 1910. On July 18, residual partial paralysis (fig. 12) of muscles supplied by right external popliteal. On this date following operation: right sciatic divided above bifurcation and immediately resutured. Then popliteals fused by usual method with two cat-gut ligatures. (The object of the division was to promote fibrillation at the cut end of the central stump, before the fibrils entered the region of fusion.) Clinically dog became worse after the operation. On December 19, 1910, nerves investigated under ether and the cut ends of the sciatic were found ununited. Yet stimulation of popliteals below neuroma evoked responses. Animal sacrificed.

Anatomic report: Popliteals in fair states of regeneration.

*Experiment 70.* Lesion March 18, 1910. On July 20 residual spastic paralysis (fig. 18) of both legs. On this date operation similar to that done on dog 65. The immediate result was flaccidity of homolateral paw. December 8, 1910, all roots which were involved in the fusion scar divided and stimulated under ether. Leg movements evoked on side of fusion, similar to those on other side. Popliteals stimulated and responded to weak currents. Animal sacrificed.

Anatomic report: At region of fusion dense scar containing partially myelinated fibers. Peripheral to scar, roots show myelination better advanced.

*Experiment 73.* Lesion March 19, 1910. On July 8, 1910, residual paralysis (fig. 24) confined to tail. On this date operation similar to that done on dog 68. In September dog was wagging tail pretty well and seemed to have more strength in it. On December 20, 1910, all roots below fusion divided and stimulated, under ether, and tail responses evoked. Animal sacrificed.

Anatomic report: Region of fusion showed dense, knotty scar infiltrated with nuclei, also a few fascicles of good fibers and some partially myelinated fibers interwoven among the cells. At more caudal levels regeneration quite advanced.

*Experiment 75.* Lesion April 12, 1910. On July 12, residual spastic paralysis (fig. 16) of right leg, except for paw which was flaccid. On this date operation on right anterior roots similar to those done in dogs 65 and 70. Clinical result completely negative. Death by accident, November 20, 1910.

Anatomic report: In region of fusion, dense scar containing some faintly myelinated fibers.

*Experiment 78.* Lesion April 15, 1910. On August 22, 1910, residual paralysis (fig. 8) chiefly of right external popliteal. On this date following operation: external popliteal divided and fused to internal popliteal low down. Dog found dead, October 10, 1910.

Anatomic report: Right sciatic above fusion contained increased connective tissue spaces. At region of fusion dense scar with numerous nuclei. New nerve fibers among these running in all directions. Some regeneration in nerves below fusion.

## *Discussion*

In the three dogs (Experiments 64, 78 and 69), in which the popliteals were fused, there was no functional gain, although there was anatomically some regeneration in all the nerves below the scar. In one of these (78) death occurred before any functional result could be expected. In dog 69 where the sciatic was sectioned higher up and the stumps failed to unite, the peripheral stump must have made new central connections through small fibers injured in the wound during the operation. The stimulation tests in Experiment 64 could not be said to be positive for the external popliteal. As to the other five dogs, the three which had their anterior roots fused on one side could not be said to show any functional gain, although two (Experiments 65 and 70) showed some response to faradic stimulation in the roots below the fusion. Anatomically again, there was some regeneration.

The only animals in which functional improvement was suggested were dogs 68 and 73, both of which had paralyzed tails, and therefore had the fusion done so as to include all the roots, taking part in the innervation of that appendage.[34] In both of these, there also were signs of good regeneration, from the anatomical and physiological points of view. However, one could not be positive that the functional return of power was due to the fusion, because it took place so soon after. To help settle this point we performed similar operations in the cauda of three normal dogs. In these cases the tails were pretty well restored in power by the end of three months. It is likely that this early improvement is owing to the shortness of route between the nerve-collectors of the tail and the roots from which these are formed.

As regards the whole question of the fusion of nerves, it is not desirable, at the present time, to discuss it further on a basis of the experiments above described, but, at some future time, after becoming acquainted with conditions of regeneration after the fusion of normal nerves, it is likely that these experiments will be alluded to again.

In closing, I wish to acknowledge the assistance of Dr. R. H. Bishop in the conduct of many of the experiments, and of Dr. David Marine in the preparation and interpretation of anatomical material. I am especially indebted to Professor George N. Stewart, Director of the Laboratory. He has made many important suggestions in the plan and details of the work, as well as in the preparation of the manuscript.

Cleveland, Ohio.

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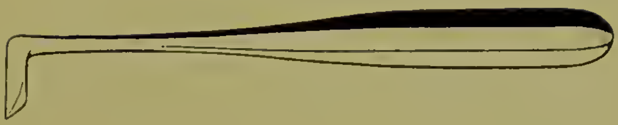
[34] Schümacher: Anat. Hefte Beiträge zur Anat. und Entwickelungsgeschichte, 120 Heft., 1909.



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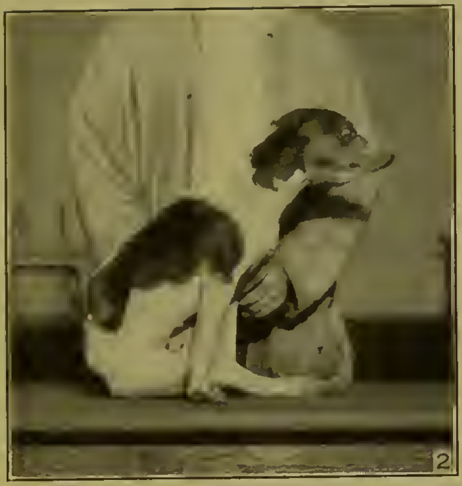
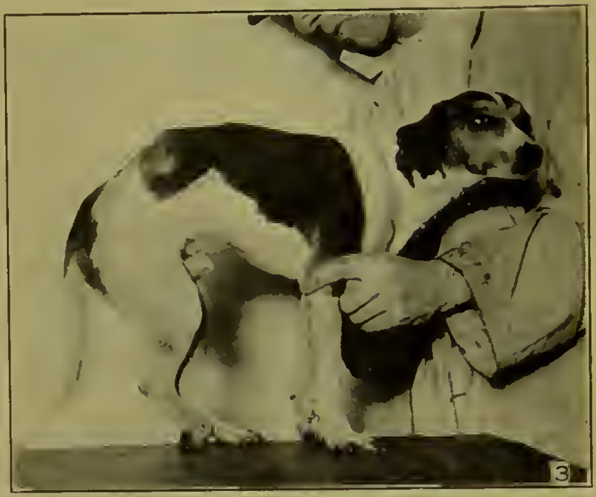
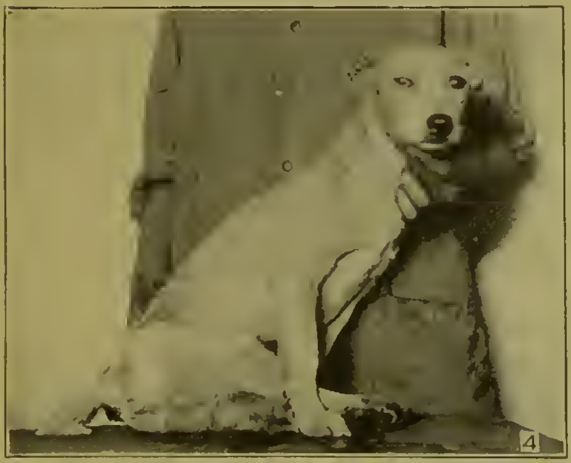






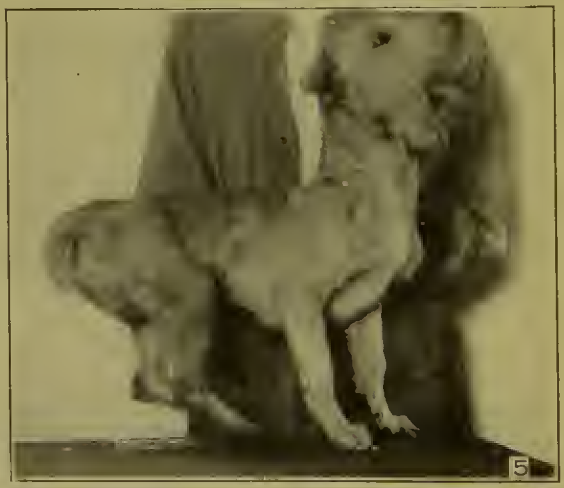
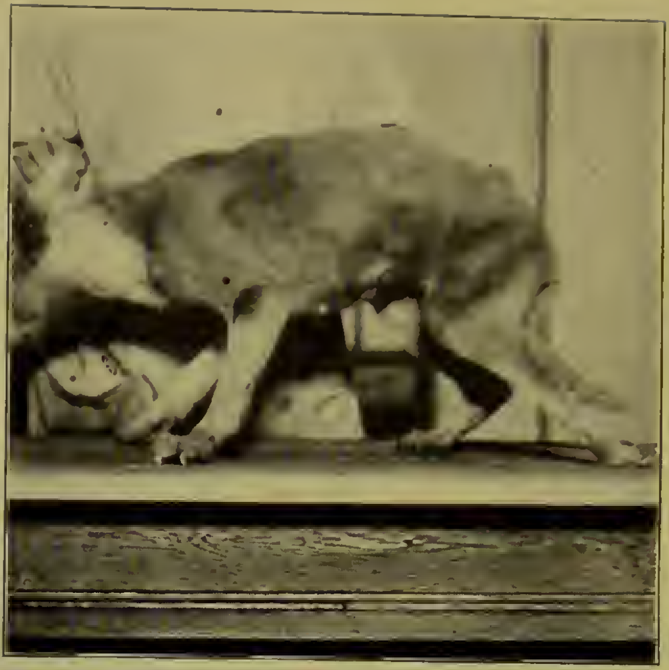


Fig. 1 Instrument used to produce lesions.
Fig. 2 Experiment 90; two days after lesion.
Fig. 3 Experiment 90; five days after lesion.
Fig. 4 Experiment 100; two days after lesion.
Fig. 5 Experiment 100; twenty-one days after lesion.



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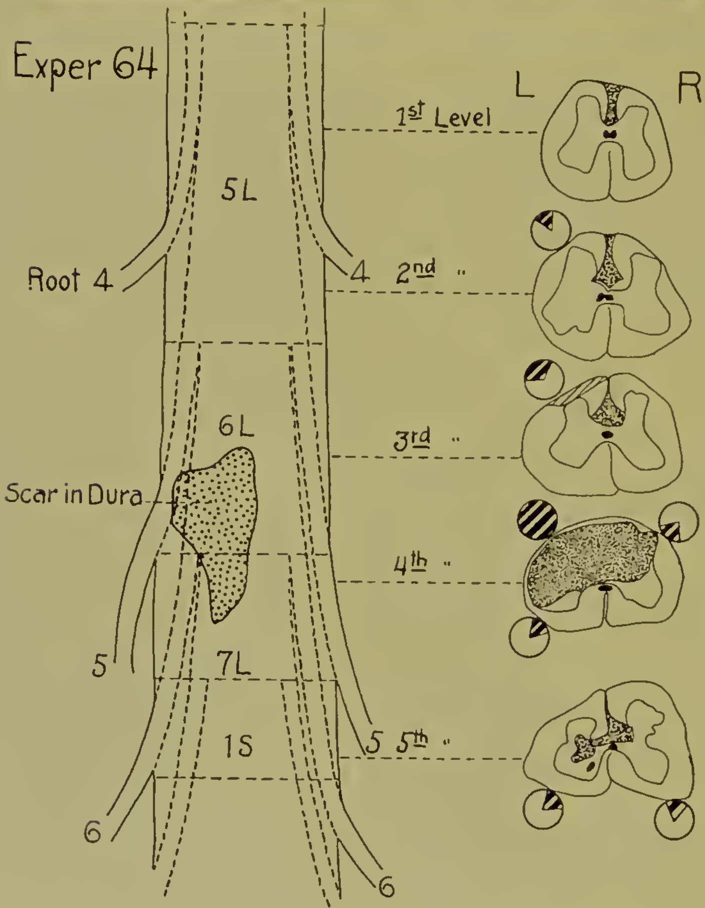

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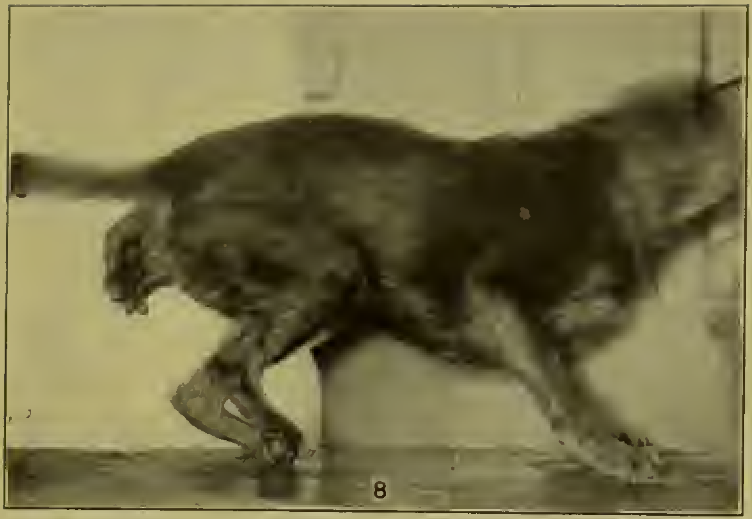
Fig. 6 Experiment 64; ninety-four days after lesion. Left, no dorsal flexion of paw and ankle; direct stimulation of nerves showed *E. P.* practically all involved.

Fig. 7 In this and the following diagrams, the topography of the cord lesions is indicated; fine stipple represents cavity; parallel line shading represents broken down tissue; cross-hatching represents dense scar from dura; the heavy shading in the circles, which represent roots, indicates the extent of involvement at the level shown; degenerations are not represented. [E. P. and I. P. = internal and external popliteals respectively.]



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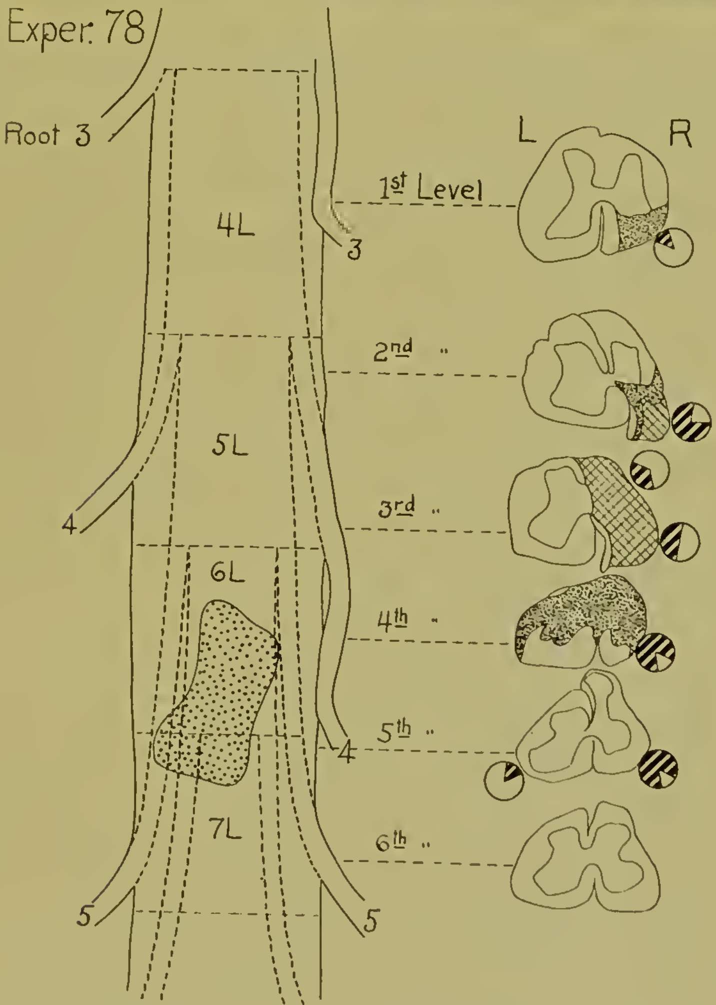

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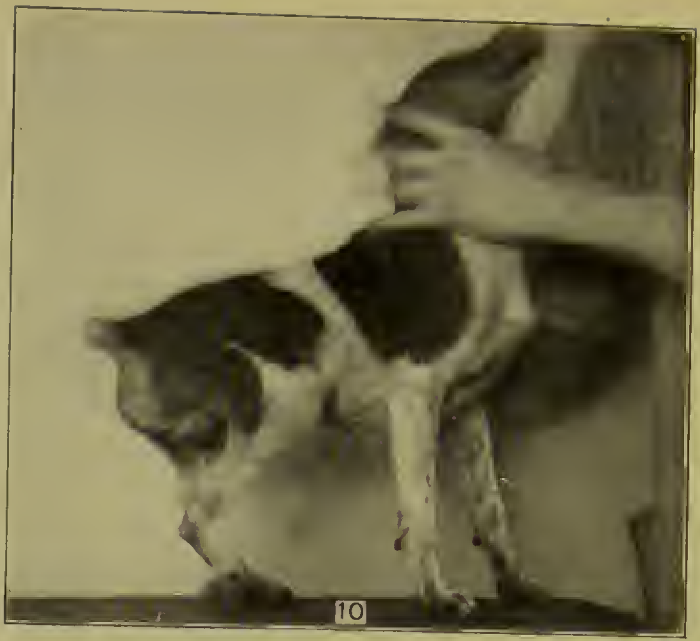
Figs. 8 and 9 Experiment 78; ninety-one days after the lesion. Right, no dorsal flexion of ankle and paw; weak extension of hip and knee; direct stimulation of nerves showed *E. P.* (right) all involved.



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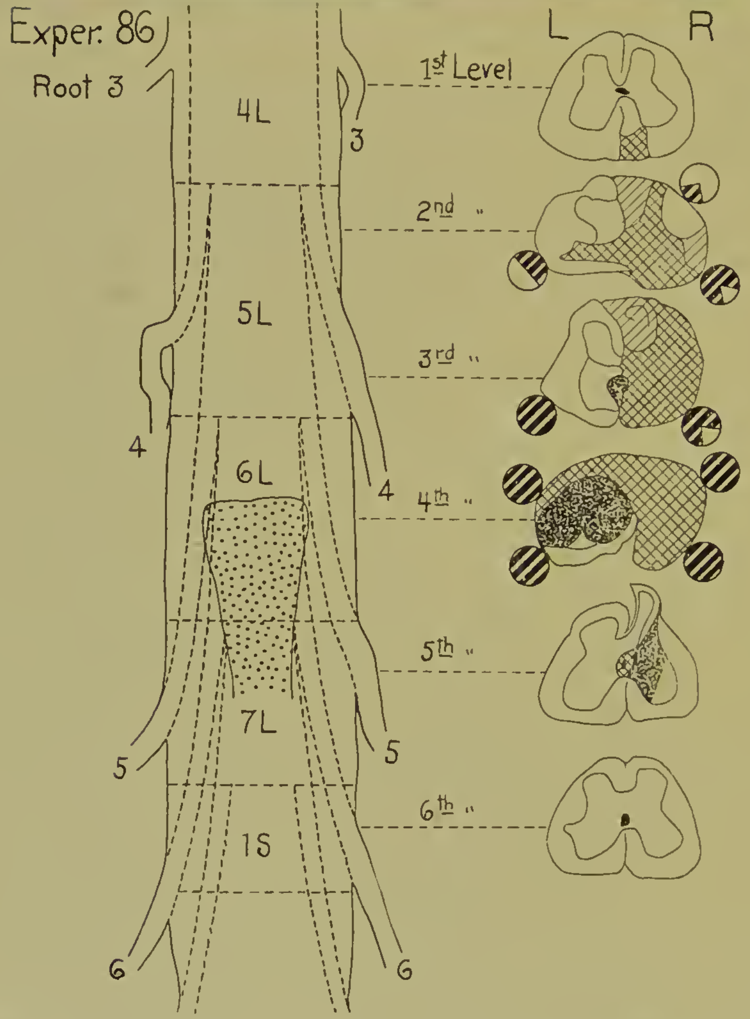

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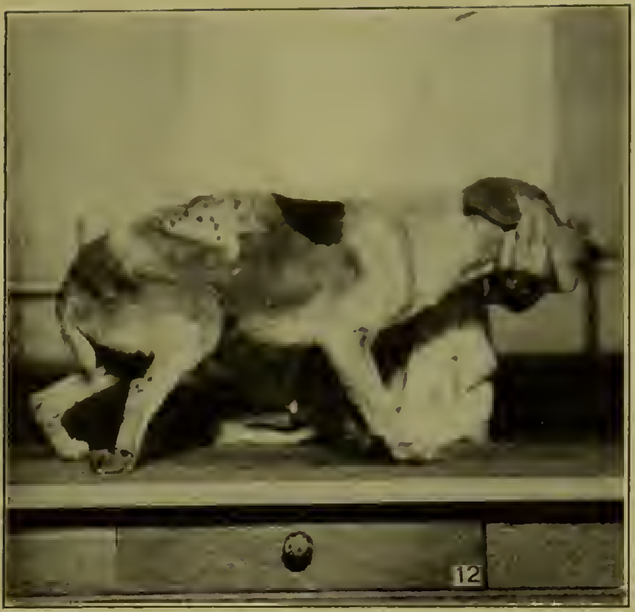
Figs. 10 and 11 Experiment 86; seventy-one days after lesion; condition little changed two to three months later, viz.: right, no dorsal flexion of paw and ankle; weak plantar flexion of toes; knee quite weak in extension; hip usually held flexed and also weak, left, stronger than right; dorsal flexion of paw and ankle gone; hip and knee slightly weak. Before killing, direct stimulation of nerves showed Right, *I. P.*, slight response in toes; no movement in ankle. *E. P.*, no response; left, *I. P.*, good response in toes; good response in ankle; *E. P.*, no response.



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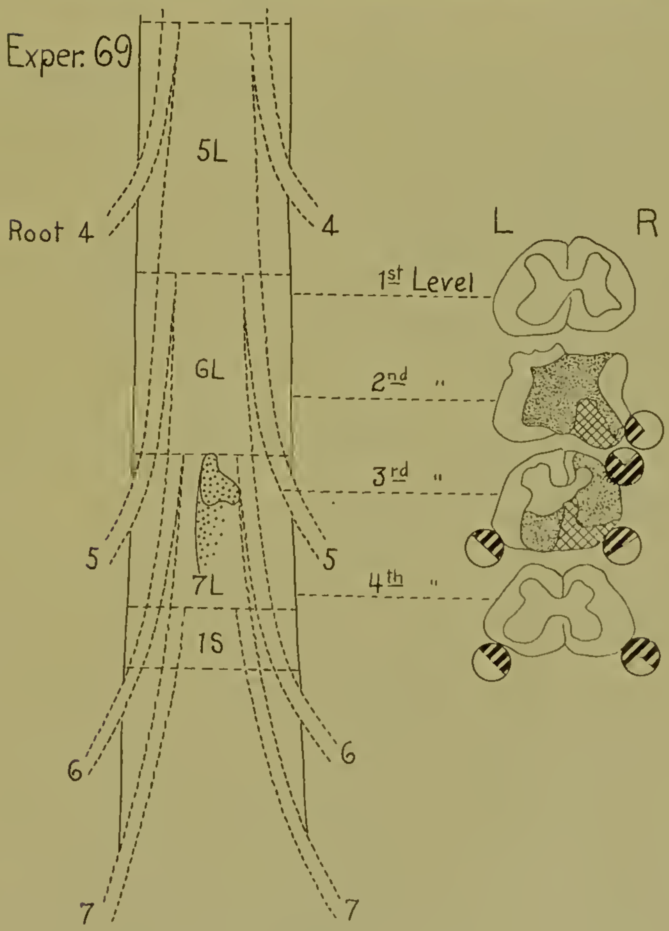

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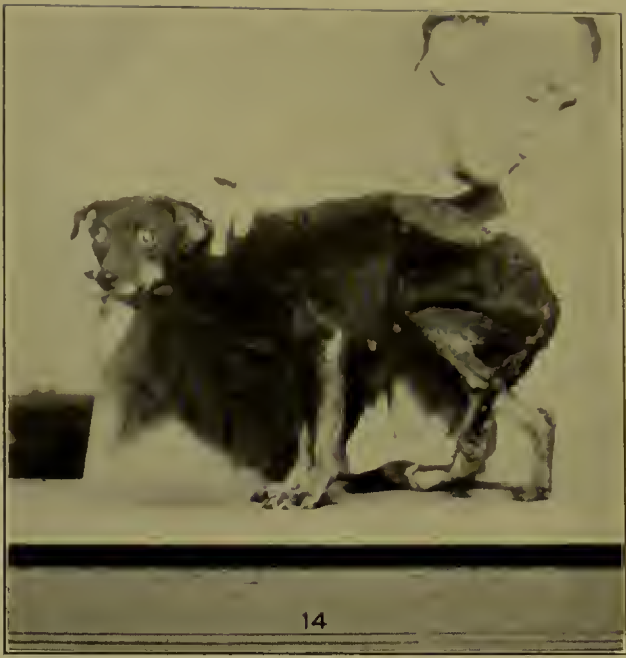
Figs. 12 and 13 Experiment 69; eighty-five days after lesion. Right, dorsal flexion of paw and ankle weak.



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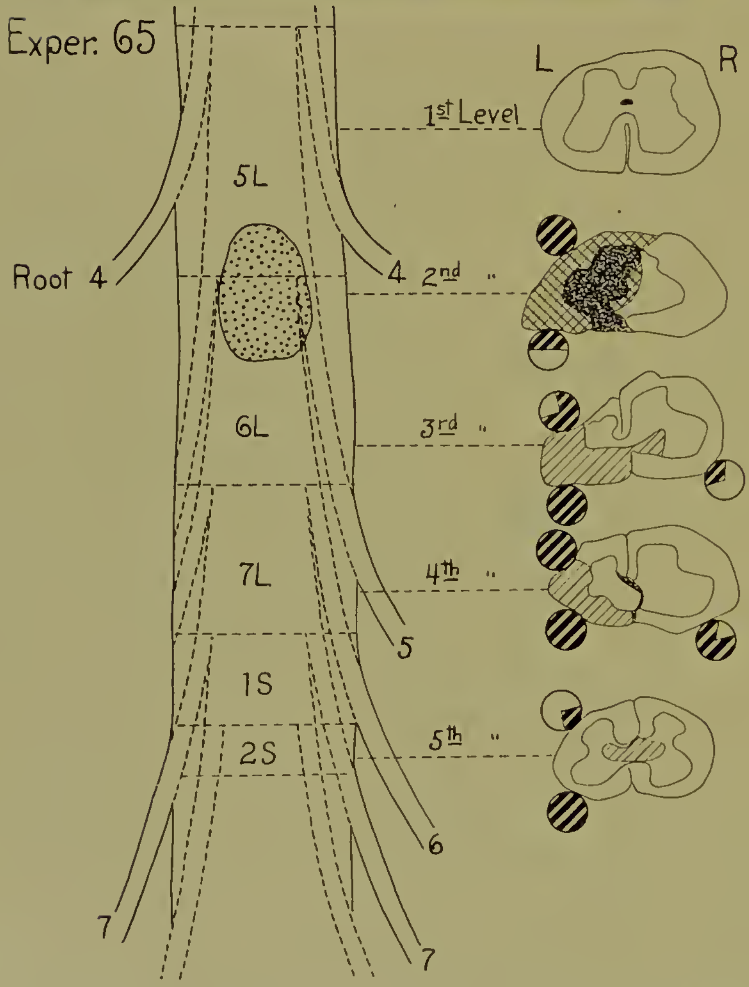

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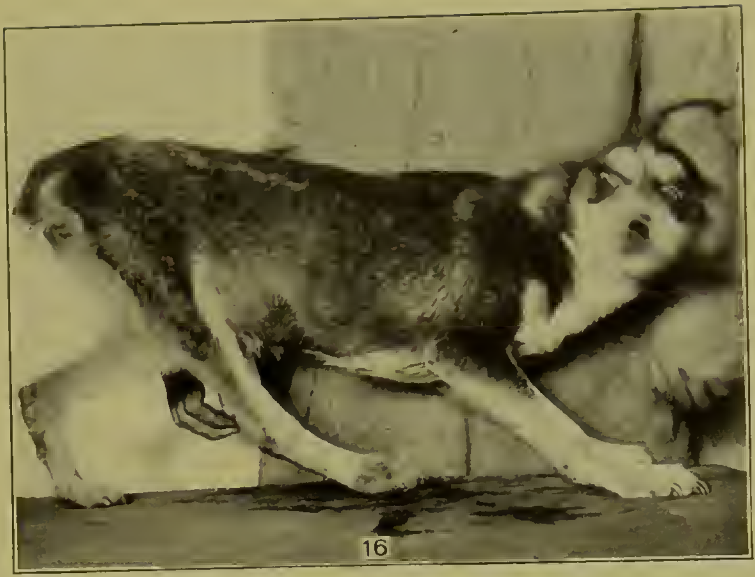
Figs. 14 and 15. Experiment 65; ninety-three days after lesion. Left, bad control of paw and ankle; knee flexed; hip contracted and extension lessened. Right, flexion of hip weak (so that she occasionally drags leg); weak power in extension of knee.



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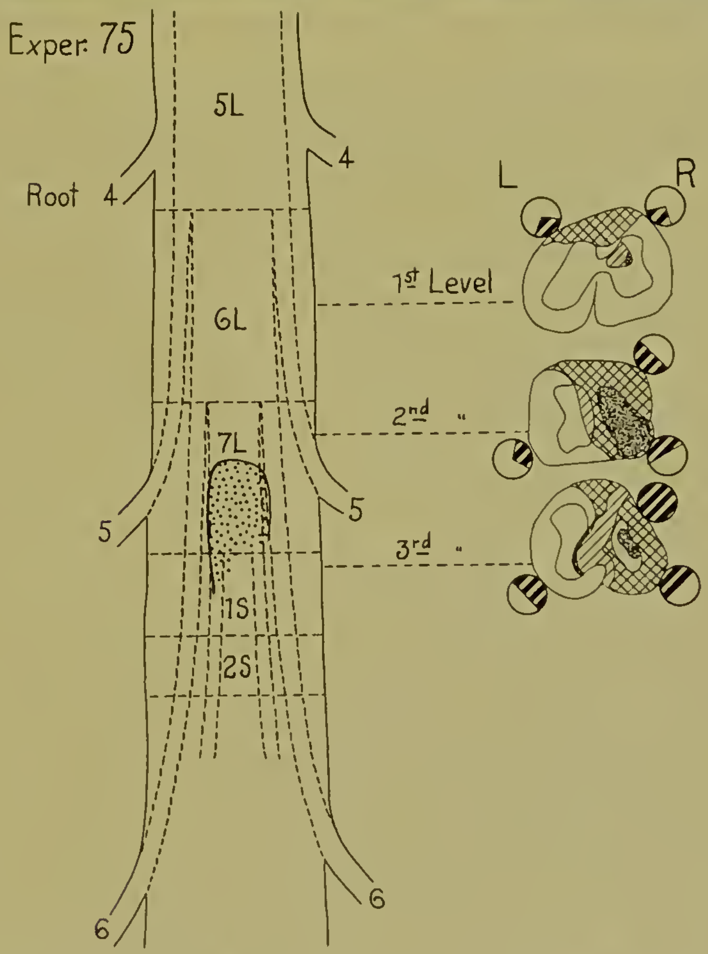

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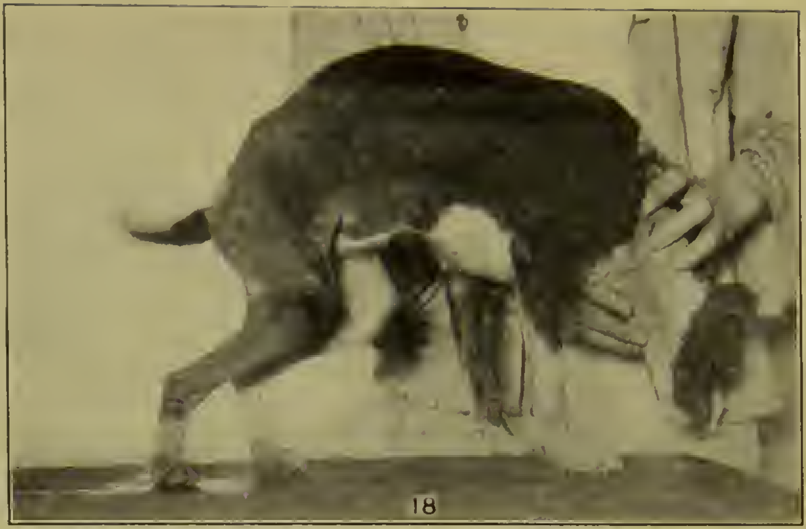
Figs. 16 and 17 Experiment 75; ninety-four days after lesion. Right, no control except in flexion of hip; leg held under her, being flexed and adducted at hip; knee and ankle held straight and stiff; paw probably paralyzed and is held flaccid; glutei and hamstrings weak; quadriceps partially gone but contracted.



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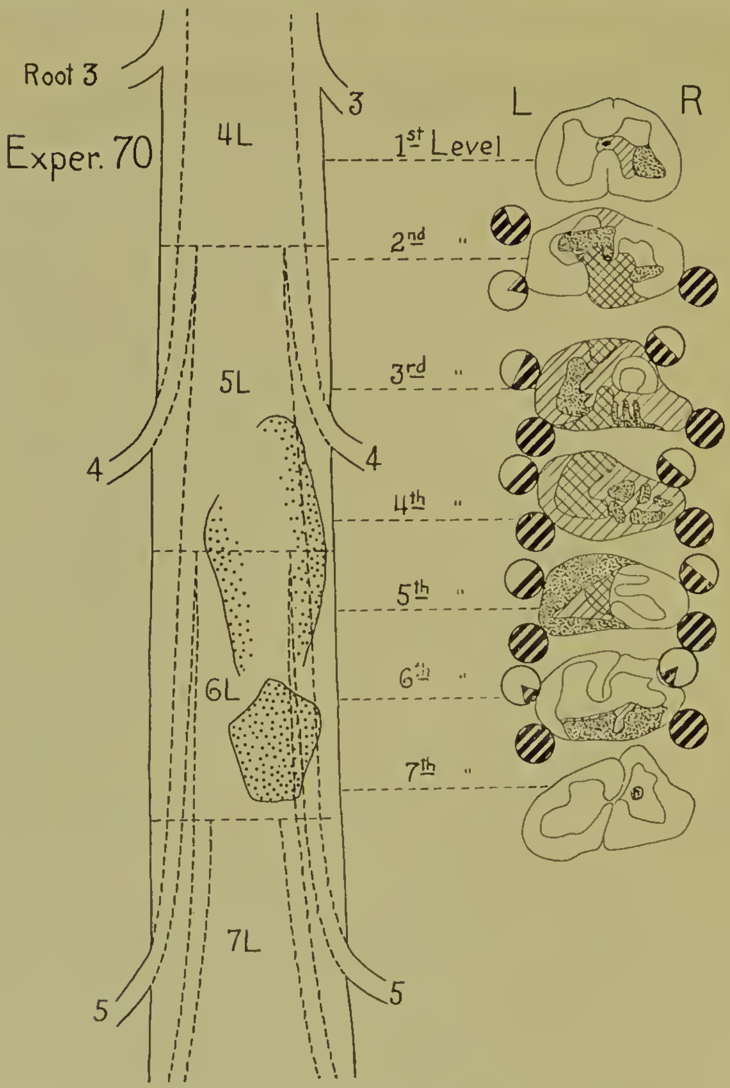

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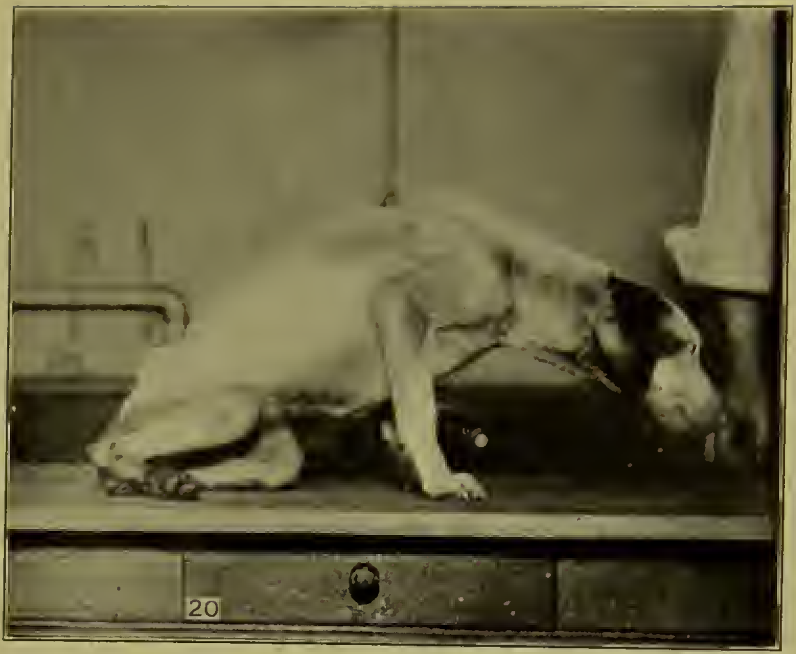
Figs. 18 and 19 Experiment 70; one hundred and twenty-three days after lesion. Poor control of pelvis, hips, knees, ankles and paws, but for the most part, not an atonic, flaccid paralysis. All joints are held permanently flexed to about normal angles, except the hips which are more extended than usual; spine bent convexly backward. On both sides erector spinae, glutei and quadriceps all weak; hamstrings fairly good; paws turn under occasionally; drags legs in position described.



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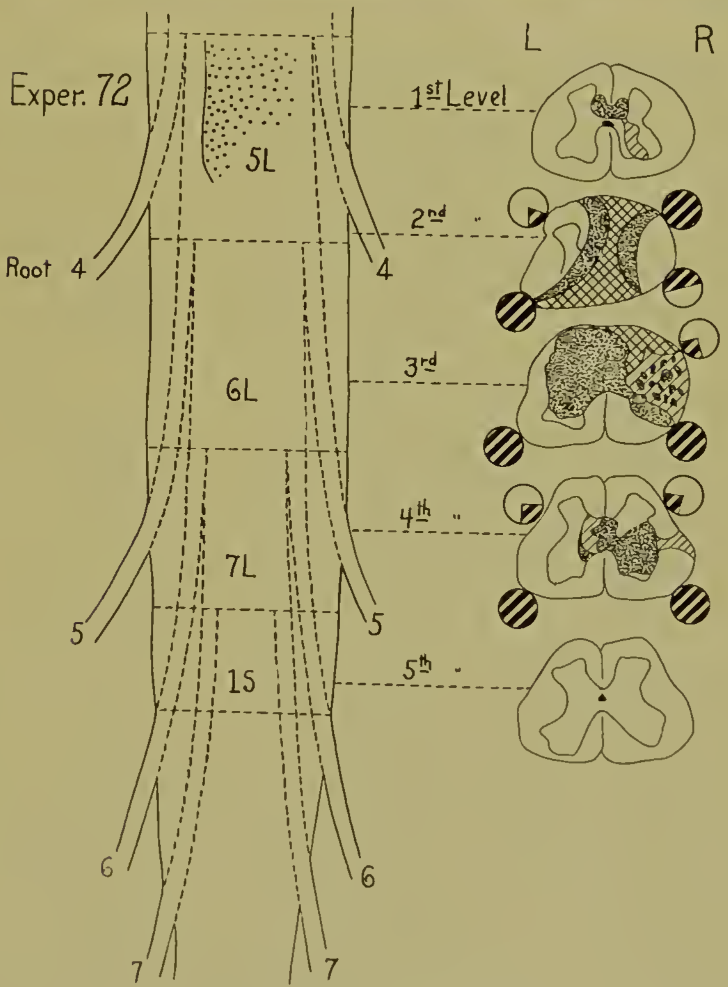

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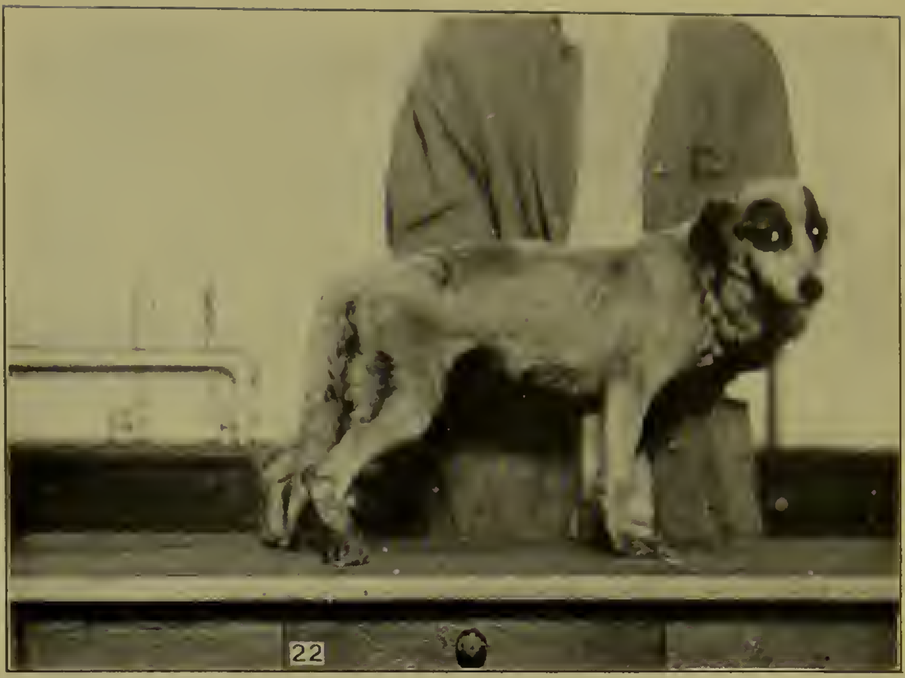
Figs. 20 and 21 Experiment 72; sixty days after lesion. Practically no control of paws, ankles, knees, hips and pelvis.



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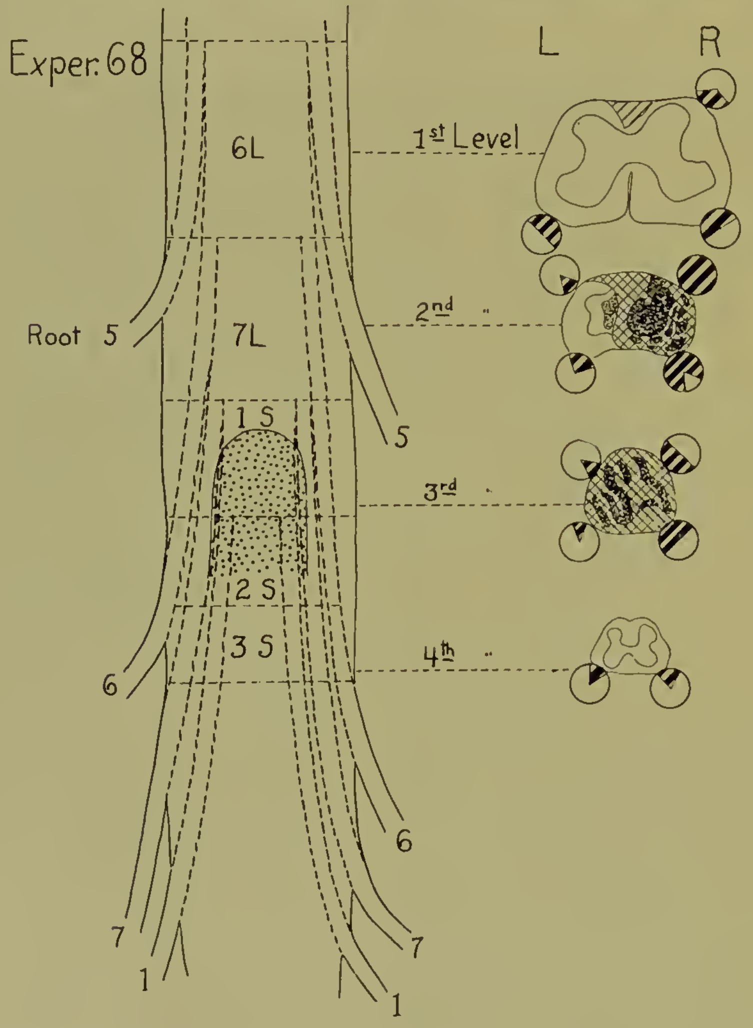

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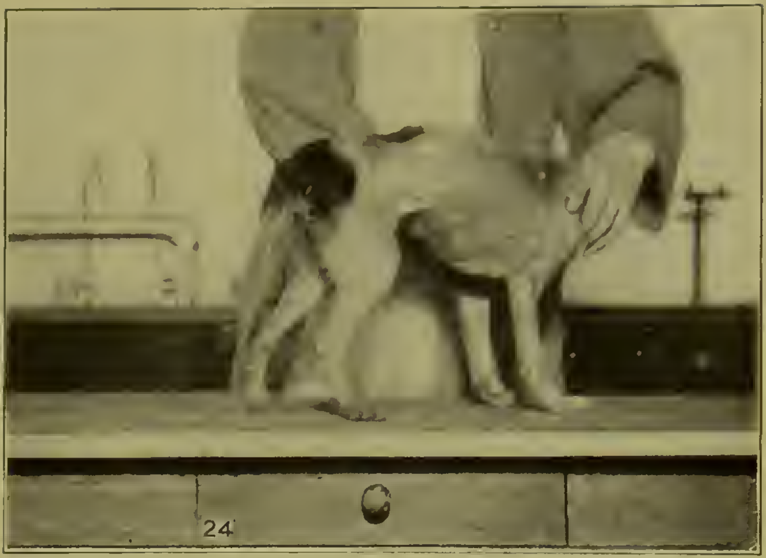
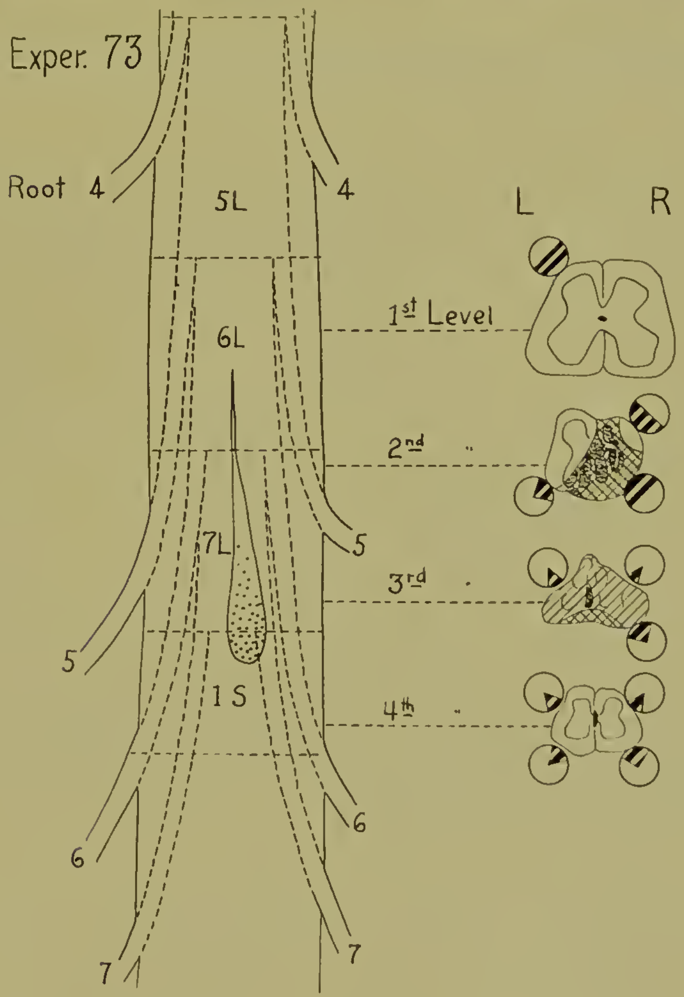
Figs. 22 and 23 Experiment 68; sixty-two days after lesion. Tail, mostly paralyzed; some power in root. Right, dorsal flexion of paw weak.





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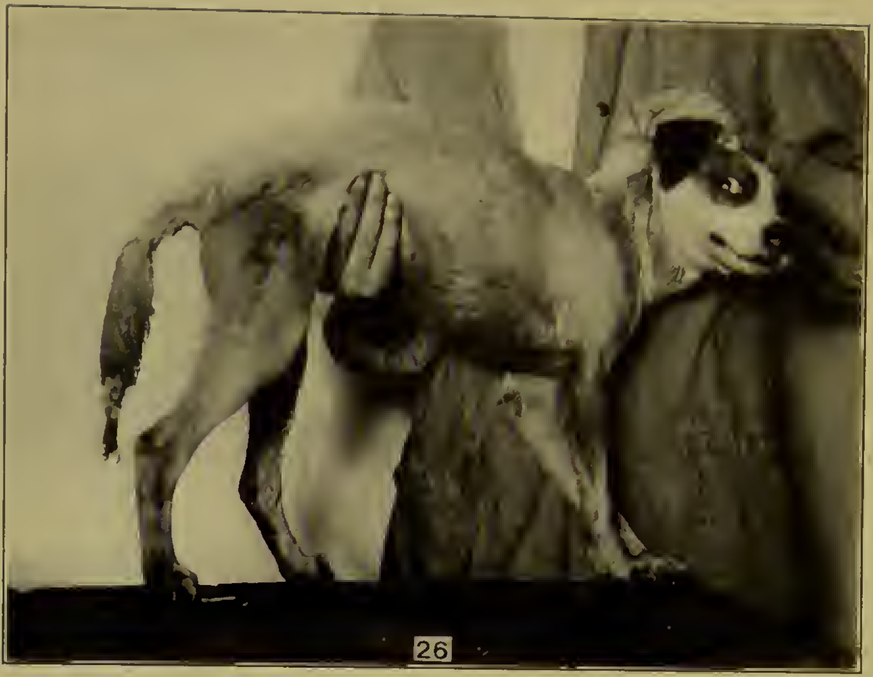
Figs. 24 and 25 Experiment 73; sixty days after lesion. Tail, mostly paralyzed; moves root a little.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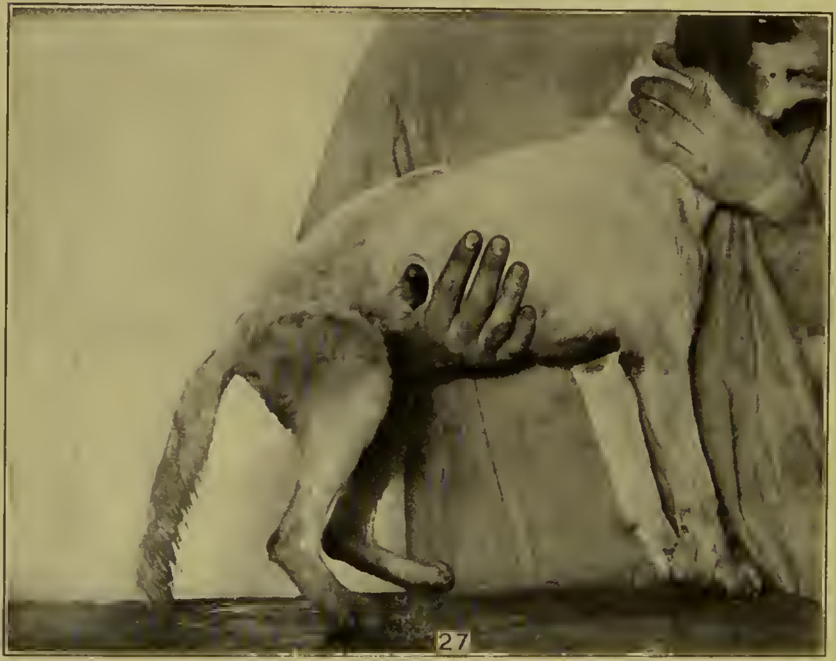
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Fig. 26 Experiment 68; one hundred and thirty-nine days after lesion; fifty-four days after fusion. Practically no control of tail.

Fig. 27 Experiment 68; two hundred and nine days after lesion; one hundred and twenty-four days after fusion; showing extent of control of tail in lifting at this date.

TABLE 1

| CLINICAL IMPROVEMENT | | | | | | HISTOLOGICAL CONDITION | |
|---|---|---|---|---|---|---|---|
| Laboratory number of dog | Number of days after lesion that autopsy was done | When first noticed | One month after lesion | Two months after lesion | Three months or more after lesion | Spinal cord | Roots and nerves |
| 71 | 10 | No improvement | | | | Mass of débris including broken down myelin and blood, which is entered by granulation tissue from pia and dura. This granulation tissue more dense toward periphery. In the interior there are young fibro blasts with elongated nuclei. Also many new capillaries. Compound granular cells, small round cells and leucocytes also present. Areas of beginning necrosis in neighboring grey matter. | Marked degeneration in some of the fibers |
| 90 | 14 | Distinct improvement noted on 5th day | | | | Similar to above. | Similar to above |
| 77 | 31 | Distinct improvement noted on 5th day | Improvement | | | Marked formation of cavity divided into compartments. Granulation tissue denser than above. Many myelin droplets left. Some fibroblasts and compound granular cells. Less débris than in above. Leucocytes and round cells in new connect. tissue. Little repair in grey matter. Some ant. horn cells swollen and pale. | Similar to above |
| 100 | 33 | Distinct improvement noted on 6th day | Improvement | | | Similar to above. | Similar to above |
| 76 | 36 | Distinct improvement noted on 11th day | Improvement | | | Similar to above. | Complete or almost complete degen. of some of the fibers |
| 66 | 50 | Distinct improvement noted on 9th day | Improvement | | | Similar to above. | Similar to above |
| 72 | 60 | No improvement | No change | No change | | Dense scar with small cavities adjoining. These contain a little débris and a few myelin drops small in size. Some small nerve fibers, perhaps new, entering cavities. Many round cells. | Similar to above |
| 61 | 63 | Distinct improvement noted on 9th day | No change | No change | | Zone of dense scar from dura. Next a zone of less well organized connect. tissue containing in its meshes compound granular cells and myelin drops. Next a zone of a relatively few fibro blasts and myelin drops, débris in large amounts and old blood. Finally normal cord substance. Some ant. horn cells are bloated. | Some remains of broken down myelin in fibers |
| 47 | 78 | Gradual improvement | Improvement | No change | | One main large cavity, which is lined by thin layer of new connect. tissue. Ant. horn cells show bloating and chromatolysis. | Some beginning regeneration |
| 42 | 112 | Gradual improvement | Improvement | Improvement | Slight improvement | Dense scar with large patches of round cells. Many leucocytes and new vessels. | Some fibers show signs of degeneration. Others suggest new regeneration |
| 86 | 159 | Gradual improvement | Improvement | Improvement | Slight improvement | Much dense scar. Two large cavities still containing the remains of myelin in small amounts.<br>Distortion of grey matter.<br>Ant. horn cells often bloated and show chromatolysis. | Similar to above |
| 78 | 178 | Distinct improvement | Improvement | Slight improvement | No change | Much dense scar. Large cavity, which tends to follow outline of grey matter.<br>Distortion of grey matter. Vacuolation of ant. horn cells. | Increase in connect. tissue in nerve trunks |
| 75 | 222 | Distinct improvement noted on 13th day | Improvement | Improvement | No change | Much dense scar containing cavities.<br>Ant. horn cells pale and bloated, having lost their polygonal shapes. | Similar to above |
| 23 | 252 | Gradual improvement | Improvement | Improvement | No change | Dense scar and cavities. Broken down tissue in grey matter.<br>Vacuolation of ant. horn cells. | Similar to above |
| 68 | 258 | Gradual improvement | Improvement | Improvement | No change | Dense scar containing cavities. | Signs of new regeneration in roots |
| 70 | 266 | Gradual improvement | Improvement | Slight improvement | Slight improvement | Dense scar, débris and light connective tissue. Cavities.<br>Distortion of grey matter. | Good regeneration of many fibers |
| 73 | 275 | Distinct improvement noted on 6th day | Improvement | Slight improvement | No change | Light connect. tissue and areas of partial necrosis in grey matter. Some dense scar with cavities. | Similar to above |
| 69 | 277 | Distinct improvement noted on 6th day | Improvement | Slight improvement | No change | 2 large cavities, one of which follows outlines of grey matter. Dense scar. | Similar to above |
| 64 | 292 | Gradual improvement | Improvement | Improvement | Slight improvement | 1 large cavity. Thick scar in dura. | Similar to above |
| 65 | 319 | Gradual improvement | Improvement | Slight improvement | Slight improvement | Dense and light scar, one cavity, débris and remains of myelin.<br>Areas of necrosis in grey matter.<br>Ant. horn cells often swollen, bloated and pale. | Similar to above |

TABLE 2

| NUMBER OF EXPERIMENT | CONTROL OF JOINTS | RESPONSE OF LEG TO POINT | RESPONSE OF PAW TO HEAT (PAIN) | REFLEXES | | SPHINCTERS | | CORD AND ROOT INVOLVEMENT (See diagrams) | POSITIVE CONCLUSIONS FOR INDIVIDUAL |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Knee jerk | Anal | Anus | Bladder | | |
| 64 | Paralysis of dorsal flex. of left paw and ankle<br>Direct stimulation evoked no response in left E. P. | Poor on both legs | No test | Absent on left | Sluggish | N | N | In upper 7th L. seg. post. horns and cols. on both sides.<br>On left, lat. col. and upper ⅔ of ant. horn. In 1st S. seg. cent. part of grey matter on left. In 5th, 6th, 7th and 1st S. seg. postero-mesial col. 5th and 6th post. roots. | Dorsal flexion of paw and ankle (E. P.), in dorsal part of ant. horn of upper 7th L. |
| 78 | Paralysis of dorsal flexion of right paw and ankle. Weak extension of rt. hip and knee<br>Direct stimulation evoked no response in right E. P. | Poor on rt. leg | Poor on right | Absent on right | Sluggish | N | N | In mid. 4th L. seg., lower part of rt. ant. horn and adjoining col. In high 5th L. seg., entire rt. ant. horn and adjoining col. In low. 5th L. seg., entire rt. half of cord except ant. mesial col. In mid. 6th L. seg., whole cord except ant. cols. and lower part of lt. lat. In upper 7th rt. ventro-mesial col. very narrow 4th, 5th and 6th rt. ant. roots. | Dorsal flexion of paw and ankle (E. P.)—in lower 6th L.<br>I. P. control lower (in 7th)<br>Extens. of hip and knee in 5th<br>Gap between centers of lower and upper leg, perhaps containing centers of hamstrings |
| 86 | Paralysis of rt. paw and ankle and of dorsal flex. of left paw and ankle. Weak extension of rt. hip and knee and some weakness of lt. hip and knee<br>Direct stimulation evoked no response in either E. P. and very slight (of toes only) in rt. I. P. | Poor on rt. leg | Poor on right | Absent on right<br>Weak on left | Sluggish | N | Weak | In upper 5th L. seg., rt. side of cord except pyram. tract, also lt. ant. horn. In low. 5th L. seg. rt. side of cord. In mid. 6th whole cord except lt. ant. col. and small part of lt. ant. horn. In upper 7th inner ⅔ of rt. ant. horn.<br>5th and part of 6th rt. and lt. ant. and post. roots and 4th rt. ant. root. | Dorsal flex. of paw and ankle (E. P.) in mid. 6th<br>Plantar flex. of paw and ankle (I. P.) in inner dorsal part of ant. horn of upper 7th<br>Extens. of hip and knee in 5th |
| 69 | Slight weakness in dorsal flex. of rt. paw and ankle | N | N | N | Sluggish | N | N | In mid. 6th all of grey matter and dorsal and ventral cols. which are partly spared on left. In upper 7th rt. ant. horn, rt. lat. col., both ant. cols. and lower ⅔ of lt. ant. horn. 6th rt. post. and 6th and 7th rt. ant. roots. | Dorsal flex. of paw and ankle (E. P.) partly in upper 7th.<br>Gap between centers of lower and upper leg |
| 65 | Paralysis of entire left leg except for flex. of hip. Weak extension of knee and weak flex. of hip on right | Poor on left | No test | Absent on left<br>Weak on right | Sluggish | N | N | In upper 6th whole left of cord, and dorso- and ventro-mesial cols. and inner part of grey matter on rt. In low. 6th lt. ant. horn and lt. antero-lat. col.; also inner part of rt. ant. horn. In low. 7th outer part of lt. ant. horn and lt. antero-lat. col. In 2d Sac. grey matter about central canal. 5th 6th, 7th and 1st lt. ant. roots; 5th and 6th lt. post. roots; 6th rt. ant. root. | Control of most of leg in 5th seg. and those below<br>Point sensation passes through 5th post. root |
| 75 | Weakness of gluteals and hamstrings on rt. hip flexed and knee straight and stiff<br>Paw paralyzed and flaccid | Poor on right | Poor on right | Absent on right | N | N | N | In mid. 6th dorsal cols. and inner part of base of rt. post. horn. In upper 7th rt. side of cord and dorsal col. and inner part of grey matter on lt. In 1st Sac. rt. side of cord and inner part of lt. ant. horn. 6th and 7th rt. post. horns. | Sciatic centers in 7th L. and 1st Sac. |
| 70 | Spastic loss of control of hind parts from pelvis down. Weakness of erector spinae, glutei and quadriceps. Hamstrings fairly good | Poor on left | N | N | N | N | N | In mid. 4th rt. ant. horn. In upper 5th lt. post. and all of antero-mesial cols. rt. ant. horn, most of lt. grey matter. In mid. 5th all of cord except rt. post. horn. In low. 5th whole cord. In high 6th lt. of cord. In mid. 6th ant. cols. and lower part of ant. horns. 4th, 5th, and part of 6th rt. and lt. ant. roots 4th lt. post. root. | Some crural and gluteal centers in upper 5th. Hamstrings below mid. 6th<br>Transection in lower 5th cuts off brain control of lower leg<br>Point sensation passes through 4th post. root<br>Heat-pain sensation above mid. 5th<br>K.J. anal reflex, control of sphincters below upper 6th |
| 72 | Flaccid paralysis of hind parts from pelvis down. | Poor on both | No test | Absent on both | No test | Weak | Weak | In mid. 5th, base of dorsal cols. part of rt. ant. root. In low. 5th, all of grey matter and dorsal and vent. cols. except left post. horn and small part of vent. col. adjoining. In mid. 6th all of grey matter and rt. ventral and lat. cols. In mid. 7th rt. ant. horn and inner part of lt. grey matter. 5th, 6th and part of 7th rt. lt. ant. roots, rt. 5th post. root. | Control of hind parts including sensation, K.J.'s and sphincters in 5th to 7th inclusive |
| 68 | Paralysis of tail and slight weakness in dorsal flex. of rt. paw. | Poor on tail<br>On legs N | Poor on tail | N | Sluggish | Weak | Weak | In mid. 6th dorso-mesial col. In mid. 7th rt. side of cord, dorso- and ventro-mesial cols. and inner part of lt. grey matter. In low 1st Sac., whole of cord (conus). 5th and 6th rt. ant. and post. roots. | Control of motion and sensation to point and heat-pain in tail in 7th L. and 1st S.<br>Anal reflex and control of sphincters in 7th L. and 1st Sac. |
| 73 | Paralysis of tail | Poor on tail | Poor on tail | N | Sluggish | N | Weak | In upper 7th, all of grey matter except lt. post. horn; also vent. cols. and lower part of rt. lat. col. and rt. dorsal col. In lower 7th entire cord (conus). | Control of motion and sensation to point and heat-pain in tail in 7th L.<br>Anal reflex and control of bladder in 7th L. |

Letter N = normal. E. P. = external popliteal. I. P. = internal popliteal

# ON THE FUSION OF NERVES.

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## I. INTRODUCTION.

Historic Sketch.—For some years I have experimented on the popliteal and other nerves of dogs, crushing and tying them together with absorbable ligatures and studying the effects. These effects have been of considerable interest. Nerves thus joined together actually grow together and may become one structure anatomically and physiologically. Not only may such nerves reassume their original function, but they may also take on increased function, as induced by the new and different combinations which are brought about by the union.

The topic presents certain aspects which seem new, and I know of no previous experiments carried out to cover the same ground. But in its analysis and experimental data it is closely allied to two important fields of research which are in turn thus brought into a new and significant relationship to each other. These two fields of research are, first, that of regeneration of nerves, and, second, that of nerve-crossing.

The anatomical data obtained from previous researches on nerve regeneration are especially pertinent to this problem. These researches have been carried out by a great many, and in this connection the works of Stroebe (1), Howell and Huber (2), Neumann (3), Bethe (4), Perroncito (5), and Poscharissky (6) are to be mentioned. These and other authors agree fairly well as to the anatomical changes presented, disagreeing materially, however, in their interpretation of the phenomena. The points of disagreement are of vital import, but need not be alluded to at the present time.

The methods previously used for observing the process of regeneration in peripheral nerves have been either by cutting and resuturing a nerve so that the stumps come into very accurate coaptation, or by using local compression, as by ligature or forceps, for a brief space of time. After either procedure the nerve below the injured portion degenerates, while a peculiar and characteristic scar forms at the place of interruption. This consists of proliferated cells and nuclei, usually conceded to originate from the neurilemma. It is important to note that these cells run in every direction. Beyond the remains of the myelin globules, which happen to be caught in this scar, nothing else is seen, even the perineuria of the nerve fascicles disappearing or blending with the mass of new cells.

This proliferation continues for some weeks, forming a swelling sometimes referred to as a "neuroma." Slowly this scar becomes organised, blood-vessels entering it from the neighbouring perineuria. New nerve fibres are now laid in the interstices of the cells, and on account of the mechanical conditions necessitated by the arrangement of the cells, these new fibres are laid down in every direction, in marked contrast to the uniform and parallel regeneration of fibres which takes place at the same time in the old nerve segment below the scar. The exact relation between the new fibres and the old ones in the central stump is the most important point in the dispute alluded to above. Be that as it may, there comes a time when the cells in the scar slowly diminish in number, the swelling disappears, and the nerve fibres tend to rearrange themselves into their original conformation and relationships.

It is not necessary at the present time to enter into the details of this process of nerve regeneration after simple interruption further than to call attention to the fact that the mechanical conditions offered by the scar are sufficient to bring about marked changes in the anatomical nerve pattern at that point, at least for the time being.

With such a premise it is but a short step to the idea, that if two or more nerves are tied together, a scar brought about by the ligature might be made to unite them so effectually as to cause fibres to pass permanently from the fascicles of one nerve into those of another. It is this that is meant by nerve-fusion, simply the tying of nerves together with the intention of obtaining a neural connection between them. But the term should include in its connotation the effect of the scar on the fibres in a single nerve-fascicle, whether or not another fascicle is joined to it.

The special condition that I originally had in mind in considering the subject was that presented in the cauda equina within the dura. By no method as yet devised could the caudal roots be sutured together so as to include a large number of them. Kilvington (7) attempted suture of the roots within the canal, but extradurally, and the best he could do was to suture one root to two others. To me it seemed that the conditions within the dura are better adapted for nerve-crossing, on account of the close proximity of a large number of important roots owing to the great obliquity of their course in this situation. An adult human preparation of a lumbo-sacral cord lying in situ within the canal with its arches removed (Quain's Anatomy, vol. iii., part 1 (1908), 11th edition, figure on p. 63) will reveal the fact that, at the level of the interspace between the dural root exits of the 1st and 2nd lumbar roots, one may gather together all the roots which go to make up the nerves supplying the lower limb, viz. the 1st lumbar to the last coccygeal roots inclusive. The condition would seem especially adapted to the method of fusion above outlined. Moreover, the intradural method permits the selection of motor roots alone. An analogous condition to that of man is to be found in the cauda of dogs. I have operated on the latter, and have found that with a moderate amount of practice the anterior roots can be gathered together and ligatured, the structures all laid back in place, the dura restitched, and the wound allowed to close tight, with no greater percentage of mortality than after ordinary laminectomies. This is not the place to describe these experiments: suffice it to state that they were carried out previous to the present ones, and afforded the indication which led me to carry out similar ones on normal peripheral nerves in order to watch the process more closely.

One question of seeming importance confronted me at the very start, viz. the best length of the scar. It was feared that too short a scar would enable the nerve fibres to rearrange themselves into their original nerve patterns. For this reason two, or sometimes three, ligatures, about $\frac{1}{8}$ inch apart, were often used in these experiments, although in a few cases one ligature only was used. Hoping to gain a better proliferation of cells, the nerves were, as a rule, also crushed with a hæmostat at the place of ligature. It was, furthermore, desirable to get rid of the ligatures after they had been in place long enough to ensure that the nerves were in a reasonably close relationship while the neuroma was forming; therefore, catgut was used.

Division of entire Subject.—The experiments so far performed may be grouped into five classes as follows:—

(1) Fusion of normal peripheral nerves in continuity (no division or excision).

(2) Nerve transplantation by fusion. (A nerve removed from its usual attachment and fused to another.)

(3) Fusion of normal spinal roots in the cauda, intradurally.
(4) Fusion of either peripheral nerves, or of caudal roots, some time after a primary cord lesion (in one case after an excision of anterior roots).
(5) Fusion of nerves partially degenerated after infantile paralysis (one human case).

A brief account of the early experiments, together with the human case of infantile paralysis mentioned in class (5), has already been published (Bost. Med. and Surg. Journ., May 11, 1911), and a summary of the experiments mentioned in class (4) is to appear shortly (Journ. of Compar. Neurol., 1912). In the present paper I will describe some of the basic experiments on normal peripheral nerves, limiting myself to the 1st and 2nd classes, viz. the fusion of nerves in continuity, and nerve transplantation by fusion.

As suggested by the researches of Osborne and Kilvington (8), the popliteal nerves were chosen for the experiments because they are easily exposed and supply antagonistic groups of muscles. The dogs used were small, full-grown mongrels. All the operations were done under complete ether anæsthesia with the usual precautions for asepsis if the dogs were to be kept alive.

Clinical Data.—On the day following the operation the animal seemed normal, except for the attitude of the operated leg. This was often held aloft, and the paw was somewhat flaccid, so that it turned under if he bore weight on it. In a few cases the limp was slight, and in some cases the leg gradually grew weaker for several weeks, when the condition reached a stage which was relatively fixed. In this stage the animal stepped on the dorsum of the paw constantly. An abrasion sometimes developed for which the only successful treatment was confinement in a kennel, the floor of which was liberally padded with shavings. Rarely the paw swelled. The abrasion presently became cicatrised and calloused, so that he was allowed to run about in the paddock for a little each day. Improvement was very slow, and not until 100 days or more after the operation did he begin to land on the planta. At the end of 125 days or so he might still turn the paw occasionally. As to the time of improvement there was much variation in different animals. Sensibility to a sharp point seemed impaired in some of the cases.

Technique.—In testing the nerves the same coil of a du Bois vertical pattern with 10,000 windings of the secondary and the core in place was always used, with the same 2-pint Daniell cell, always filled to the same level, in the primary circuit. The stimulation was bipolar, the electrodes being fastened in a 2-inch rubber handle. In some of the experiments the current was roughly measured by noting the distance of the secondary from the primary, as shown by the scale on the side of the slide. At 125–150 mm., as read on that scale, one could begin to taste the current on the tongue, and if not otherwise stated, this was the strength of current usually used. The nerves were freed for as long a distance as possible before they were stimulated, which was done both before and after division. The nerves were always ligatured before they were divided, and by means of the ligatures were carefully held suspended as far out of the wound as possible, well away from other structures. In the earlier experiments no special note was taken of the effects of mechanical stimulation, but in the later ones these were carefully noted. Usually it was sufficient for this purpose to note the effects of the ligatures when they were drawn tight.

The terms "dorsal" and "plantar" flexion are used instead of the terms "extension" and "flexion." The abbreviations "EP" and "IP" refer to the external and internal popliteals respectively.

All the nerves and scars were studied anatomically. Before placing the preparations in hardening fluids, they were stitched on stiff cardboard, in a condition of slight tension. Each nerve was labelled on the mounting board, together with the date and number of the experiment. A sketch was made to accompany each specimen. Later, after proper fixation, small pieces were removed for imbedding and section, each piece having been properly labelled and numbered and its place marked with a corresponding number on the sketch. All these precautions were necessary to prevent confusion.

The stains used were various, depending on what was essentially to be studied in the individual case. Chief reliance was placed on sections stained for myelin sheaths by the method of Weigert-Pal. Occasionally these sections were counterstained with Upson's carmine. The general stains used were the hæmatoxylin-eosin and the Van Giesen. To show regeneration of axis-cylinders in the scar, the methods of Stroebe and Yamagiva were most admirable. The silver impregnation of Ramon Cajal was applied in four cases (with two failures). To show fresh degeneration after secondary excision the Marchi method was used. Both celloidin and paraffin were used for imbedding.

For nerve counts Weigert-Pal sections were used. The counts were made by means of a camera-lucida, as follows: The fascicle, or a definite portion thereof, was first outlined on a sheet of paper and then, with a pen dipped in indian ink, in the right hand, each fibre was crossed off on the paper, the crossing marks being plain enough to be seen under the microscope. As each nerve was crossed off it was counted at the same time, using for this purpose a so-called "tallying register" held in the left hand and worked with the thumb. By this method there was no possibility of counting any fibre more than once, the chief source of error being the difficulty of counting the small fibres and those cut obliquely.

## II. Fusion of Nerves in Continuity (fig. 1).

There were nine experiments in this class. In seven of these, physiological tests were applied, but in the other two the dogs were dead before the opportunities for such tests presented themselves.

### (a) Short-Interval Experiments.

Exp. 105—August 3, 1910—Ether. Operation.—Fusion of popliteals of left leg by crushing them together with hæmostat and tying with one catgut ligature. Dog limped till death, which occurred September 17, 1910. (45th day.) Cause of death not determinable.

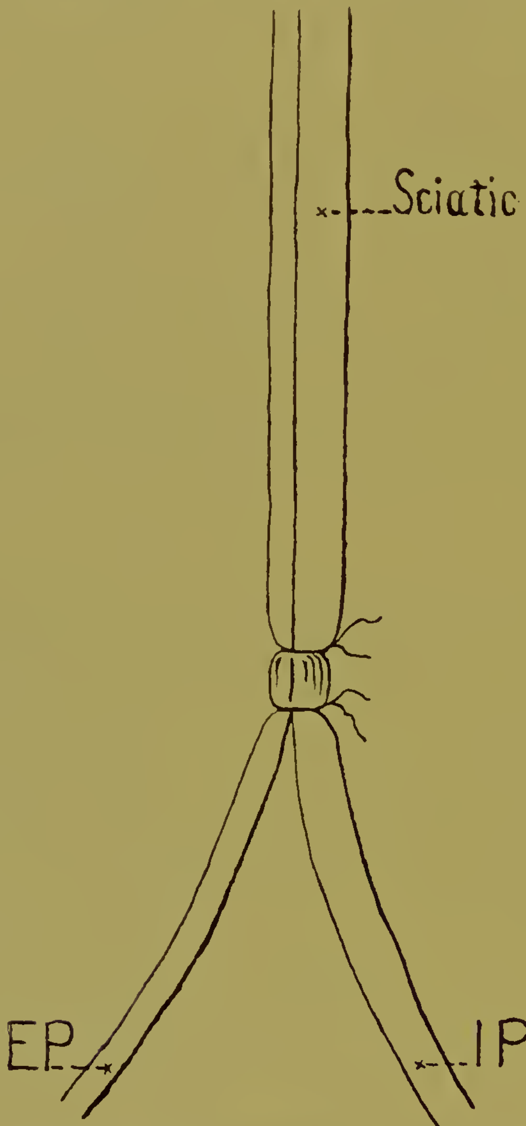


FIG. 1.—Diagram to show usual method of fusion in continuity.
EP—external popliteal.
IP—internal popliteal.

Anatomical Findings.—Left popliteal nerves found joined by small neuroma. Longitudinal sections through this showed no place where the perineurium of any nerve fascicle was broken, or where nerve fibres entered the surrounding scar. In the nerve fascicles one could make out several zones in the following descending order:—1st, a zone where the fibres presented end-bulbs; 2nd, a zone of brush-like terminations and a few spirals; 3rd, a zone of small fibres with large interspaces, which was the condition of the fibres in the peripheral segment. It was plain that the nerves were undergoing an active state of regeneration.

In marked contrast is the next case, Exp. 106, in which the fusion was done at the same sitting and in exactly the same way as the previous one, using one ligature of catgut and crushing the nerves with a hæmostat. The animal lived about the same length of time (56 days), and was killed by another dog with which he got into a fight while getting his exercise in the paddock. He limped until time of death. At autopsy the popliteals were found joined by a neuroma as in the other case. Histological sections, however, showed marked differences; fascicles from both popliteal nerves entered a scar consisting of a mass of cells containing vast numbers of nuclei closely packed together (fig. 2). The cells were

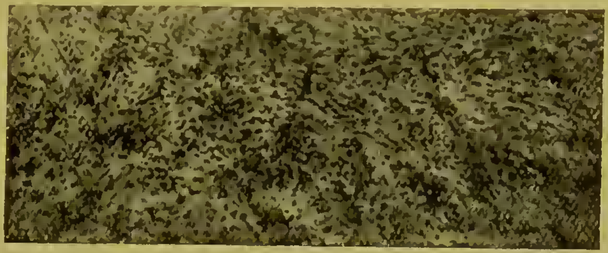


Fig. 2.—Exp. 106. Section of scar in neuroma 56 days after fusion, showing the marked proliferation of nuclei. (Hæmatoxylin and eosin stain. Enlargement, 80 diam.)

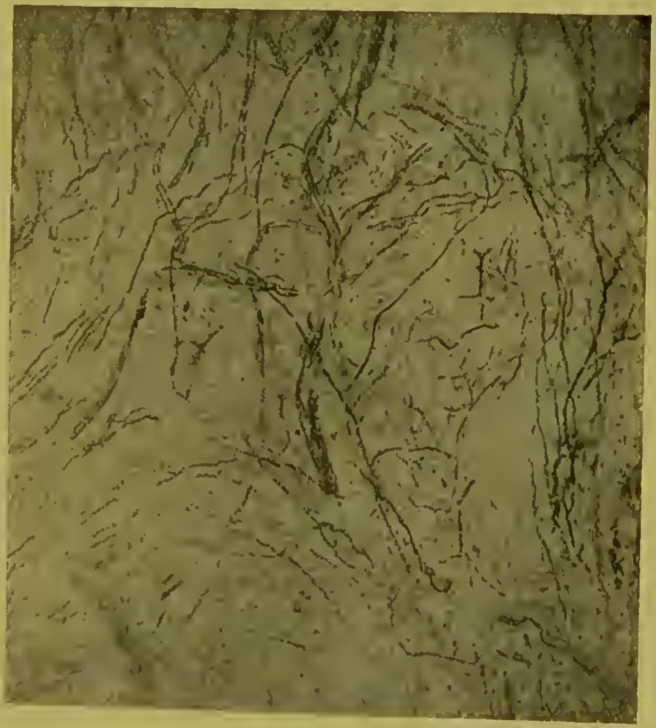


Fig. 3.—Exp. 106. Section of same tissue as that of fig. 2, showing the crossing of fibres in the scar. (Weigert-Pal stain. Enlargement, 80 diam.)

arranged in knots and clumps without reference to the direction of the nerve fascicles. There were blood-vessels, but no perineurium was to be distinguished except as blending with the scar. Laid down in the interstices of these cells were nerve fibres in various stages of regeneration. These (fig. 3) ran in all directions as necessitated by the cells among which they formed, i.e. upwards, downwards, transversely, diagonally, and crossing each other frequently. Fibres from the external side of the scar passed into the IP and vice versa. Some fibres were seen to pass directly from one popliteal to the other. As they passed out of the scar they became rearranged into straight and parallel paths to enter the external and internal popliteals which they now constituted. The direction of fibres is illustrated in fig. 4. Before entering the scar many of the fibres seemed to break up into globules and often presented delicate fibrillated ends like brushes, and occasionally a spiral.

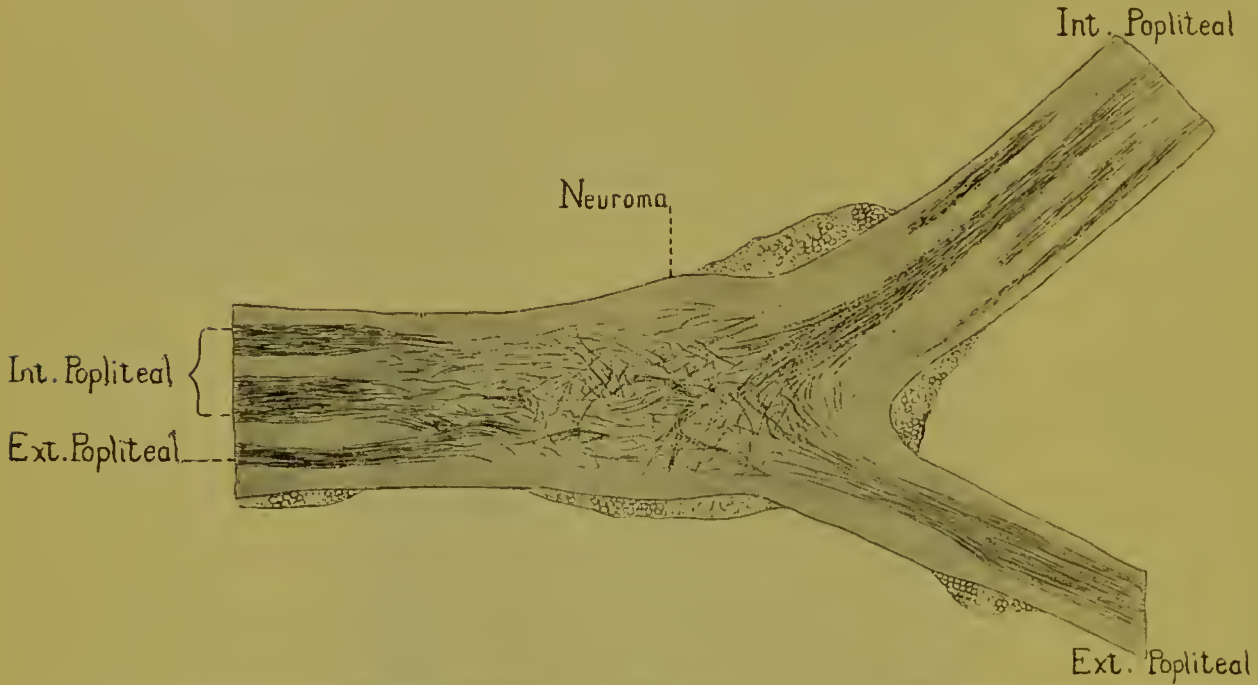


Fig. 4.—Exp. 106. Semidiagrammatic representation of longitudinal section through neuroma with popliteal nerves attached. (Drawing made from Weigert-Pal preparation, with dissecting microscope. Enlargement, 5 diam.)

We have here distinct anatomical evidence for several important points: 1st, that the direction of the new nerve fibres in the scar depends on the arrangement of cells of which the scar is formed; 2nd, that the result is at least a partial crossing of the fibres; and 3rd, that the old tracts in the peripheral segments are not necessarily entered by the fibres which originally occupied them. I can offer no reason why this condition should prevail in the latter case and not in the previous one.

## (b) Long-Interval Experiments.

Exp. 125—March 10, 1911—Ether. Operation.—Fusion of right popliteals, using two ligatures of catgut $\frac{1}{8}$ inch apart. Dog limped until time of killing, May 30 (81 days), when popliteals were again exposed and tests applied. On this date nerves found fused for $\frac{3}{16}$ to $\frac{1}{4}$ inch, being joined by a whitish, well-defined neuroma slightly thicker than sciatic. Faradic stimulation evoked good responses in both nerves below the neuroma, but there was no passage of impulses through it.

> Anatomical Findings.—Neuroma hardened in alcohol (95 per cent.) and successfully impregnated with silver by the Cajal method. Sections cut serially and counterstained with safranin. It was found that, starting from above the neuroma, many of the nerve fibres formed long and barbed growth-bulbs ("cones de croissance" of Cajal) or broke up into end-brushes (neuro-fibrils). The fibres and fibrils entering the scar became entwined, crossed, and twisted among each other and often formed large and intricate spirals as described by Perroncito (5). Many of the neuro-fibrils seemed true branches of others. At one place (figs. 5, 6, and 7) a collection of cells with fibres in the interstices boldly crossed another similar collection. This crossing occurred in several layers, some fibres intermingling with others and other fibres turning at right angles in their own course to enter the other collection. There were large numbers of nuclei staining deeply with safranin and presenting granular ground substances and well-defined limiting membranes. Nucleoli were rare or indistinct.

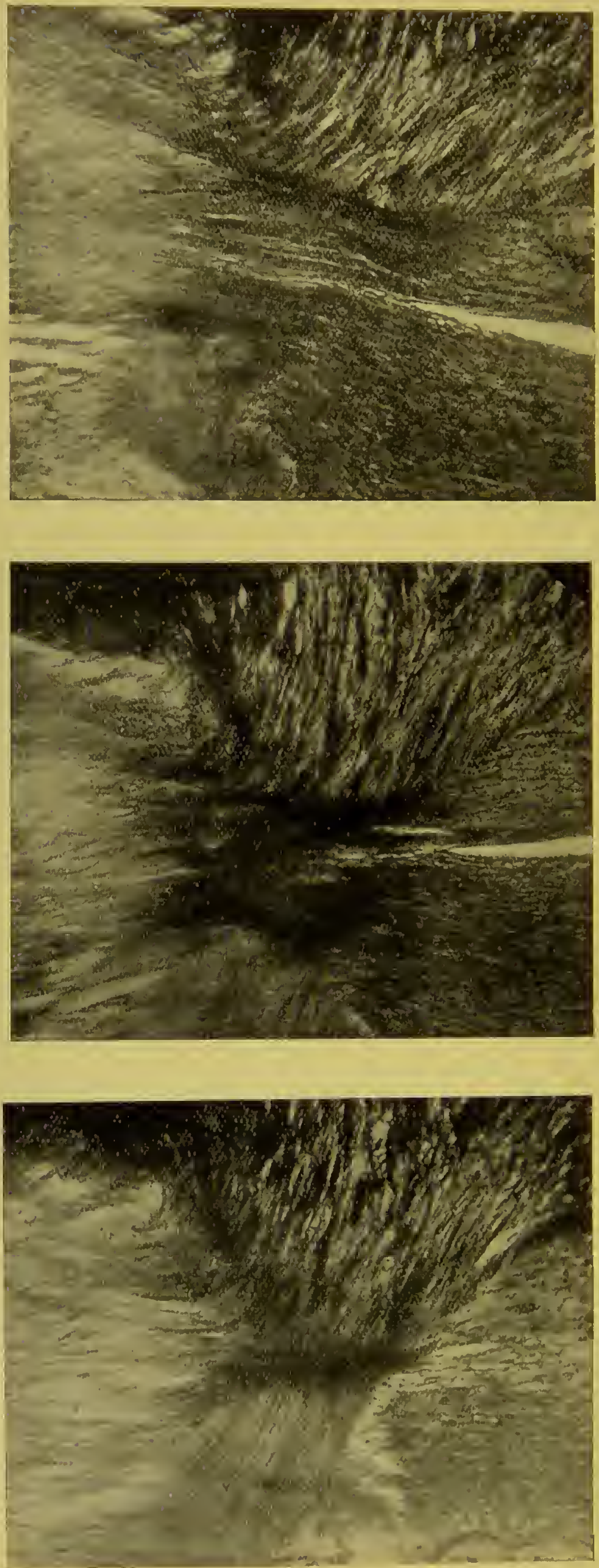
The next two experiments presented somewhat different pictures, at least from the physiological point of view.

Exp. 110—September 23, 1910—Ether. Operation.—Fusion of right popliteals by crushing with hæmostat and tying with two catgut ligatures $\frac{1}{8}$ inch apart. Usual limp. October 24, 1910 (21 days later), nerves again exposed under ether in order to ascertain whether or not there was complete loss of faradic excitability in the nerves directly after the fusion and before regeneration had fairly begun. The two nerves were found to join in a round bulb, slightly thicker than the sciatic, and from which a slender gray strand ran peripherally and ended in the two popliteals (fig. 8). All structures touched with the electrodes, and all failed to respond.

February 1, 1911 (131 days after fusion), dog still lame. Paw not sensitive to squeezing (Perroncito's test). Nerves again exposed under ether and found to be joined by the usual neuroma, the slender gray strand having become filled out. Stimulation results:—

Before division:
Stim. of IP above neuroma—dorsal or plantar flex. of ankle and paw.
„ EP „ „ „ „ „ „ „
„ IP below „ „ „ „ „ „
„ EP „ „ „ „ „ „ „

After division of EP above neuroma:
Stim. of peripheral stump of same—dorsal flex. of paw and ankle.



Figs. 5, 6, and 7.—Exp. 125. Sections selected from a series illustrating the crossing of dense collections of fibres in the scar, in several layers, 81 days after fusion. (This tissue was impregnated by the silver method of Ramon Cajal. Enlargement, 80 diam.)

After division of IP below neuroma:
Stim. of peripheral stump of EP—dorsal flex. of paw and ankle.
„ central „ IP— „ „ ” ”
„ undivided IP above neuroma— „ ” ”

After division of IP above neuroma (this left only the peripheral EP undivided):
Stim. of periph. stump of IP above neuroma—dorsal flex. of paw and ankle.
” ” „ EP ” ” ” ” ” ”
” ” ” IP below ” ” ” ” ”

Division of EP below neuroma (specimen removed):
Stimulation of peripheral stump of EP left in leg—dorsal flexion.
” ” ” IP ” ” plantar „

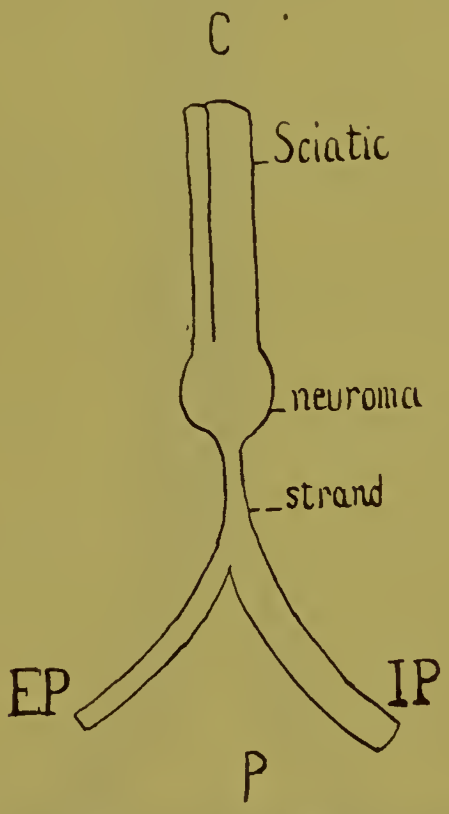


Fig. 8.—Exp. 110. Diagram representing conformation of popliteal nerves 21 days after fusion.

These tests would suggest that impulses passed through the scar both along the original courses of the nerves as well as from one to the other. I believed at the time that I had taken sufficient precautions to rule out spread of current by freeing long pieces of nerve and by only using currents of moderate strength. Later, however, as a precaution, a preparation was made of normal nerves, something like the above preparation, when both nerves had been freed and all segments cut except the peripheral EP below the scar. The normal preparation consisted simply of a piece of the excised IP tied to the EP just firmly enough so as not to break the perineuria and of about the same length as the two IP stumps above and below the neuroma in the case of the fusion. The tie corresponded to the neuroma. On stimulating the excised nerve even at its ends, some response was evoked in the muscles supplied by the other, although the minimal stimulus necessary to evoke the response had to be increased according to the distance from the tie.

Therefore, in both the above and in another experiment (Exp. 111) which, except for the time of the tests (102 days), offered precisely the same data as the above, escape was not absolutely ruled out, the only thing that was certain being the fact that regeneration was very good below the neuroma.

The anatomical findings in these two cases were similar to those of Exp. 105. The zones of growth-bulbs and end-brushes were very distinct, and marked crossing of the fibres in the scar could be noted in many places.

Nerve-counts were made in Exp. 111 with the following results:—

The IP above fusion contained 10,494 fibres.
„ IP below „ „ 9,699 „

This was a decrease of 795 fibres, or 7·5 per cent. (Some of the IP fascicles were damaged below the neuroma, perhaps explaining part of the decrease.)

The EP above fusion contained 5,028 fibres.
„ EP below „ „ 5,252 „

This was an apparent increase of 224 fibres, or 4·4 per cent. I say apparent because, on account of the close crowding of normal fibres, it is very possible that some of the smaller ones might have escaped notice in the upper segments. The fibres in the lower segments, though smaller than the upper ones, had large interspaces. At any rate, it was plain that most of the sheaths were refilled.

On account of the failure to rule out escape of current completely, in interpreting stimulation tests in the previous experiments, not having made the control experiment described above, greater care was used in the remaining experiments, and observations were also made on the effects of mechanical stimulation. Moreover, no evidence has been presented as yet to show that the crossing of fibres, as anatomically demonstrable, persisted long enough to seem permanent. In the present series the longest interval allowed to supervene after the fusion operation was about six months, and this experiment is presented next.

Exp. 115—November 30, 1910—Ether. Operation.—Fusion of right popliteals, using two catgut ligatures $\frac{1}{8}$ inch apart and crushing the nerves with a hæmostat. Dog limped till two months or so before killing, which occurred May 21, 1911 (172 days after fusion). On this date nerves exposed again and found joined by the usual neuroma (fig. 9). Stimulation tests resulted as follows:—

Right leg—before division:
Above neuroma IP—plantar flex. paw and ankle.
EP—dorsal ,, ,, ,,
Below neuroma IP—plantar ,, ,, ,,
EP—dorsal ,, ,, ,,

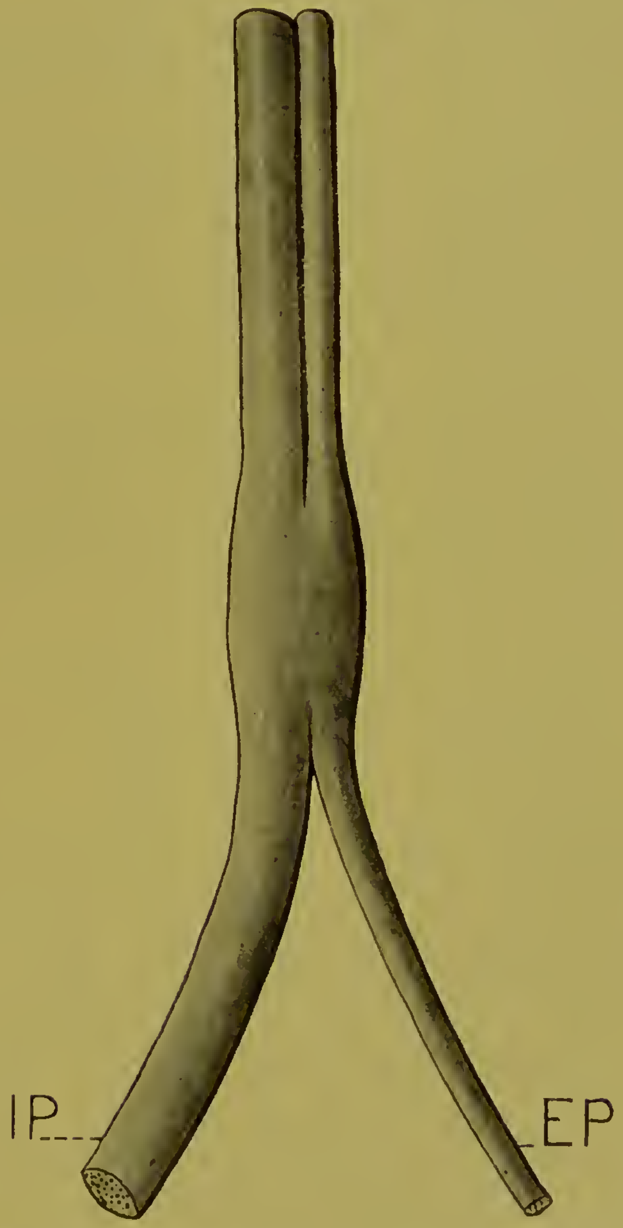


Fig. 9.—Exp. 115. Semidiagrammatic representation of popliteal nerves 172 days after fusion.

IP below neuroma tied with two ligatures, one of which evoked plantar flexion of paw.
IP divided between ligatures.
Stim. central stump—dorsal flex. paw and ankle.
,, other nerves above and below neuroma evoked same response.

EP tied above neuroma, evoking dorsal flexion of paw.
EP divided above ligature.
Stim. periph. stump of same evoked dorsal flex. of paw and ankle.
" central " " IP now evoked flexion of knee.
IP tied and divided above neuroma—the tie evoked flexion of knee (probably through small branch to biceps).
Stim. of both stumps of IP above and below neuroma now evoked dorsal flexion of paw and ankle, and the minimum stimulus required to evoke response was the same all along the nerve's course.
EP more sensitive than IP both above and below neuroma. Mechanical stimulus applied to IP stumps leading to and from neuroma now completely negative.

Stimulation of stumps left in leg with minimal currents necessary to evoke responses resulted as follows:—

| | |
|---|---|
| Right—EP 370[1] | IP 250 |
| Left—EP 385 (limit of coil) | IP 385 (limit of coil) |

Anatomical findings showed about the same condition as that found in earlier scars. There was the usual mixture of fibres and a rather large number of end-brushes. It was plain that the fibres frequently crossed, thus suggesting that this crossing remained permanent.

Nerve-counts:—

| | | | | |
|---|---|---|---|---|
| IP above | fusion | contained | 9,778 | fibres. |
| IP below | " | " | 11,434 | " |
| EP above | " | " | 3,995 | " |
| EP below | " | " | 4,545 | " |

This was equivalent to 16·9 per cent. increase for the IP and 13·7 per cent. increase for the EP. As in Exps. 110 and 111, the regenerated nerve fibres in the lower stumps were small and the interspaces large.

The experiment, therefore, furnished some evidence for the passage of impulses through the scar, both from one nerve to the other, as well as along the original course of one of the nerves, the important points being, 1st, the response of the muscles supplied by the EP when it was mechanically stimulated above the neuroma; and 2nd, the response of the muscles supplied by the same nerve (EP) when the IP was stimulated with faradism above and below the neuroma, escape being ruled out by the fact that the minimal current necessary to evoke response was the same wherever along the course of the nerve the electrodes were touched. The effects of minimal stimulation suggested almost normal return of power as compared with the normal leg. Comparing the nerve-count with that in Exp. 110, one might be led to believe that the difference in the length of time after fusion might explain the difference in the number of fibres, but two experiments are not sufficient to settle this point.

---

[1] Numbers represent position of secondary coil, as read in millimetres on side of inductorium.

### (c) Long-Interval Experiments. Secondary Excision above Neuroma.

If fusion of two nerves brings about a neural connection between them, the question arises, what is the effect of a secondary interruption of one of the nerves above the scar? The following experiments were done to answer this question, and show most interesting results.

Exp. 116—December 10, 1906—Ether. Operation.—Fusion of popliteals of right leg using 2 catgut ligatures ⅛ inch apart. On May 19, 1911 (160 days after fusion), dog no longer lame, and on this date popliteals again exposed (under ether) in the right leg. Sciatic separated into its EP and IP divisions above the neuroma. IP ligatured ¾ inch above neuroma. Drawing ligature tight evoked plantar flexion of paw. Stimulation of peripheral stump of same with faradic current evoked sharp dorsal flexion of ankle and extension of toes. Ligatured end of IP, including about ½ inch of its length, now cut off (again causing plantar

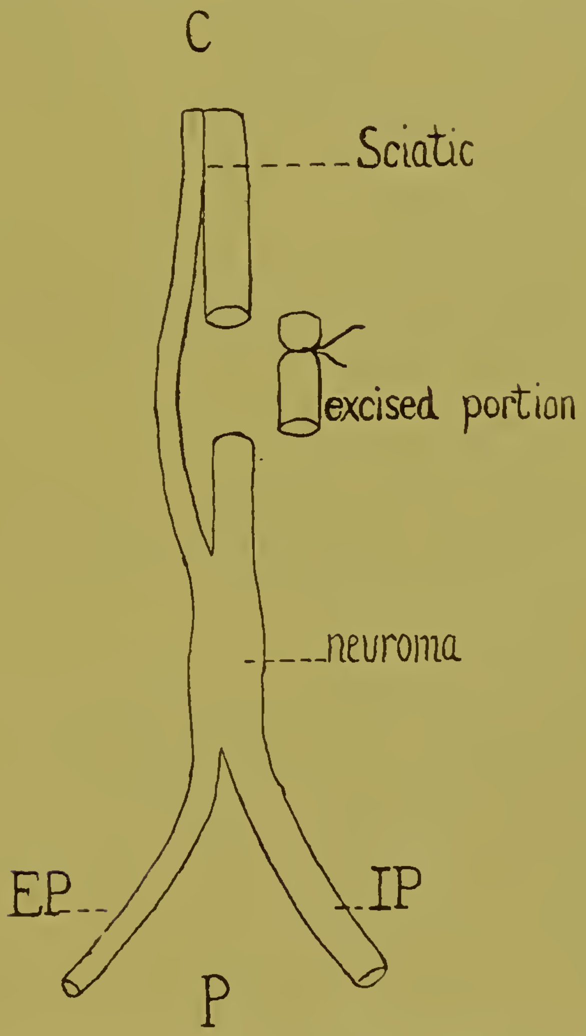


Fig. 10.—Exp. 116. Diagram to show place where the IP nerve was excised 160 days after fusion and 16 days before autopsy.

flexion of paw). The conformation of the nerves and the location of the excised portion are indicated in fig. 10.

Similar excision of IP in left (normal) leg as a control.

After the dog recovered from the immediate effects of the operation he seemed lamer than before on his right leg, his ankle, knee, and hip flexing too sharply when he lifted it. The paw did not turn under. Left leg seemed practically normal.

June 4, 1911 (sixteen days after the excision and 176 days after the fusion), right popliteals again exposed (under ether), and dissection showed a new bridge of tissue, where the piece of the IP had been excised. All nerves freed and IP separated from EP above neuroma without disturbing new bridge of tissue connecting the IP stumps.

Stimulation before division :—

IP above neuroma and above excision bridge—flex. of knee (probably through branch).

IP above neuroma and below excision bridge—evoked no response.

IP below ,, plantar flex. of paw and ankle.

EP above ,, ,, ,, ,, ,, ,,

EP below ,, dorsal ,, ,, ,, ,,

EP divided below neuroma between two ligatures. Tying of ligatures evoked no response. Stim. central stump evoked no response.

IP divided above excision bridge, evoking no response. Stim. of undivided EP above fusion—evoked flex. of knee and plantar flex. of paw and ankle.

EP divided above neuroma. Stim. of periph. stump of same—evoked plantar flex. of ankle. Stim. of lower stump of same (lower EP attached to neuroma) now evoked plantar flex. of ankle.

Stim. of neuroma itself—evoked strong plantar flex.

,, IP. stump above neuroma again evoked no response.

IP divided below neuroma and specimen removed. Both IP and EP stumps left in leg responded well to both faradic and mechanical stimulation.

Dissection on left (control) leg shows similar bridge of tissue between IP stumps.

Stimulation before division :—

IP above excision—slight dorsal flex. (must be due to escape of current, for new nerves could not have developed in 16 days).

Stim. of IP below gap—no response. IP divided.

EP responded well before and after division.

Stim. of nerve stumps left in legs with minimal currents sufficient to evoke response :—

Right—EP 200 IP 250
Left—EP 370 IP 0

These stimulation tests on the right leg seemed to show that, so far as could be judged by such tests, after a piece had been excised from the IP, degeneration proceeded as far down as the scar, and here seemed to stop, as was evidenced by the fact that the stumps of both popliteals left in the leg strongly reacted to the same tests.

Anatomic Findings.—Specimens of each nerve above and below neuroma stained by Marchi method. The IP below the excision gap and above the neuroma showed practically complete degeneration. The EP above the neuroma seemed normal. The latter showed some fatty elements, which, as pointed out by Weigert (9), are to be expected with the usual Marchi reaction in normal peripheral nerves. The sections were, moreover, exactly similar to those of the EP on the other leg, which was surely normal. Below the neuroma, however, there were scarcely any signs of degeneration either in the EP or the IP, the condition seeming exactly alike in the two, while the total amount of degeneration in them appeared nothing like as much as that seen in the IP above the neuroma and below the excision. A cross-section through the neuroma also showed but little degeneration. All these sections were controlled by Weigert sections from the same locations, and although a few degeneration products could be made out in the scar, and below it, the great majority of fibres had the usual characteristics of fibres seen in that particular stage of development (176 days).

Most interesting were longitudinal sections through both halves of the neuroma. The upper half was cut serially, and showed the EP and IP fascicles entering the scar. The latter were seen to be in an extreme state of degeneration and, ending fan-like in the scar, they presented a marked contrast to the EP fascicles and other parts of the sections where there was but little fat. The condition is pictured in fig. 11. Sections of the lower half of the neuroma showed the two nerves leaving the scar, presenting but an extremely small number of fatty particles, perhaps less than normal.

The above experiment seems very suggestive in showing the crossing of fibres in the scar. Here a nerve fused to another failed to degenerate below the place of fusion after it had had a piece of it excised above that place. Yet, previous to that excision, impulses set up by both electrical and mechanical stimuli had been conveyed from the upper segment of the nerve through the scar and into the lower segment.

A second experiment (Exp. 120) was done according to the same general plan and showed similar results. In this, however, the EP instead of the IP was excised. This excision was done 152 days after the fusion. At this date stimulation of the EP, both above and below the neuroma, evoked normal responses, but stimulation of the upper IP evoked dorsal flexion. After the operation the dog limped but did not turn his paw under on either leg (a control operation having been done on the other side). Twelve days later (164 days after fusion) the responses elicited by stimulation were, as in Exp. 116, very good in all the nerve segments except, of course, in the segment of the EP below the excision gap and above the neuroma. If degeneration took place below the neuroma, in either nerve, it was certainly not complete. Histologically, the Marchi reactions corresponded to those in Exp. 116, except for one important point, viz., that there were more fatty particles in both lower segments—enough, in fact, to suggest a moderate degeneration. As it was the EP that was excised in this case, and this nerve is much smaller than the IP, the sum-total of fatty elements in the lower segments, although only moderate, might very well have been equivalent to those in the EP above the neuroma.

But in a third experiment (Exp. 117), done exactly as Exp. 116, the findings were different. In this the IP was also secondarily excised 160 days after fusion, and the results of stimulation at this date were precisely the same as those obtained in Exp. 116. Seventeen days later, however, the findings were radically different. The IP failed to respond completely both below as well as above the neuroma. The EP responded fairly well to physiological tests, both above and below the neuroma.

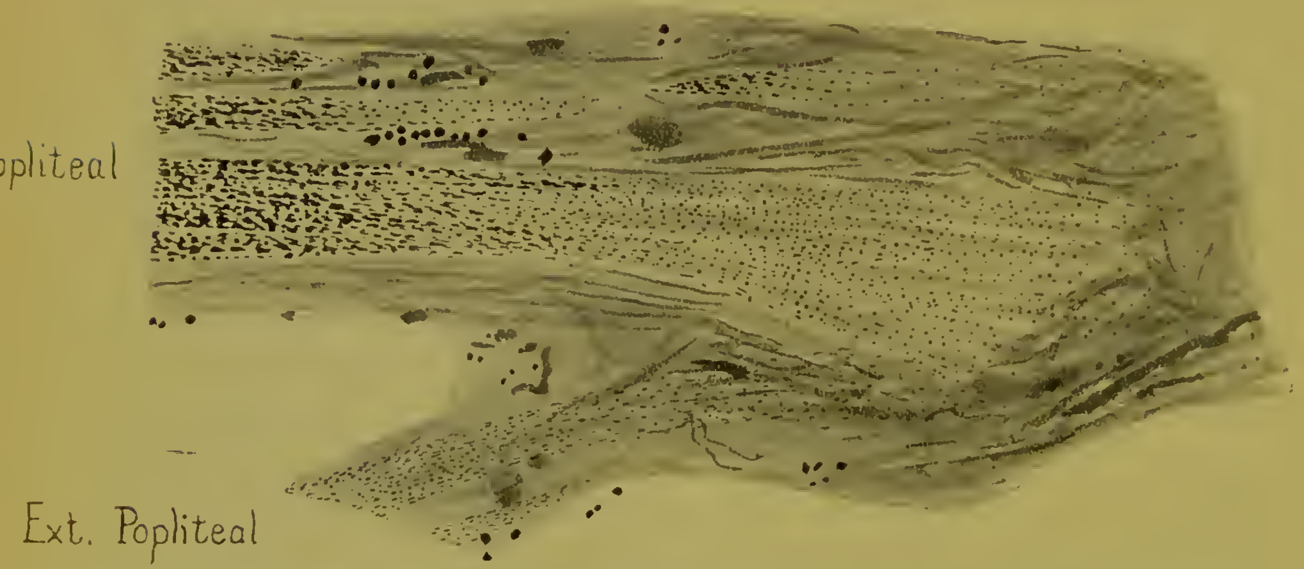


Fig. 11.—Exp. 116. Semidiagrammatic representation of longitudinal section through central half of neuroma, with popliteal nerves attached, 176 days after fusion. The IP had a piece excised 16 days previously (see fig. 10). (Drawing made from Marchi preparations; with dissecting microscope. Enlargement, 12 diam.)

The Marchi sections, however, did not seem to bear out these physiological differences in the two nerves; there seemed to be about as much degeneration in the lower segment of the EP as in that of the IP. If there was a difference, it was slight. Not only this, but the amount of degeneration seemed to be about the same as that to be seen in the corresponding segments in Exp. 116, and this means that there was surely less fatty deposit than in corresponding segments in Exp. 120, which, the reader will recall, did respond to physiological tests.

The physiological and anatomical (Marchi) reactions of the nerve segments below the neuroma in these three experiments may be compared in the following table :—

| | Reaction of segments below neuroma. | |
|---|---|---|
| | Physiological tests. | Marchi reaction. |
| Exp. 116 IP (excised above neuroma) | Good response. | Very slight. |
| EP | " " | " " |
| Exp. 120 IP | " " | Moderate. |
| EP (excised above neuroma) | " " | " |
| Exp. 117 IP (excised above neuroma) | No response. | Very slight. |
| EP | Good response. | " " |

There are, therefore, several points that require elucidation; 1st, that after secondary excision of a nerve fused to another, signs of degeneration may fail to appear below the scar of fusion; 2nd, that if this is the case in one experiment, it may not be so in another, although the two might deliberately be carried out according to precisely the same plan; and 3rd, that the signs of degeneration in the lower segments, as manifested physiologically, may not be borne out by clear signs of degeneration anatomically, if the Marchi method is used.

Is there any explanation by which these seemingly contradictory observations may be reconciled to each other as well as to accepted notions of nerve degeneration and regeneration?

As is well known, there are two chief theories for the regeneration of nerves after division—one that of autogenetic regeneration, and the other the outgrowth theory. According to the former theory, regeneration of the nerve fibre is peripheral; according to the latter it is from a central source only. There are a number of theories which take intermediate grounds, and according to which both the central and peripheral stumps take part in the formation of new fibres.

It might seem that the observations above noted are more readily interpreted according to the theory of autogenetic regeneration, or some of the theories related to it, than according to the outgrowth theory. Thus it is easy to see that if the nerve fibres in the lower stumps regenerated, after the original fusion operation, with little or no aid from the central stumps, then one might expect to find some such results after secondary excision above the scar, as are actually to be noted in these experiments.

Economy of space prevents my entering further into this subject as bearing on the above observations. Suffice it to say that the arguments against such an interpretation are simply the arguments against autogenetic regeneration, and these I believe are so strong, as set forth by Langley and Anderson (10), Ramon Cajal (11), Ross Harrison (12), and others, that for the present at least I will try to explain these results in accordance with the usually accepted notions, viz. the classical outgrowth theory.

Adhering therefore to the outgrowth theory, the first explanation which seems worthy of consideration is that before the nerve was excised the responses evoked by physiological tests below the neuroma depended on a very few fibres only (for it is known that a nerve will react very well even to crude physiological tests when only a few fibres in it are intact), so that after excision the Marchi reaction would hardly be evident on account of this very scarcity of degenerated fibres. This would explain in Exp. 116 the close resemblance to each other of the Marchi reactions in the two lower segments as well as their resemblance to the corresponding segments in Exp. 117, and would still be consistent with the more striking picture in Exp. 120. But the difficult thing to comprehend according to that explanation is the fact that in Exps. 116 and 117 the nerves which had been subject to excision above the neuroma seemed to show a far greater number of degenerated fibres than both stumps together below the neuroma. What became of the remainder? The only possibility is that they all made connections with the neighbouring tissues through scar adhesions. But this is unlikely.

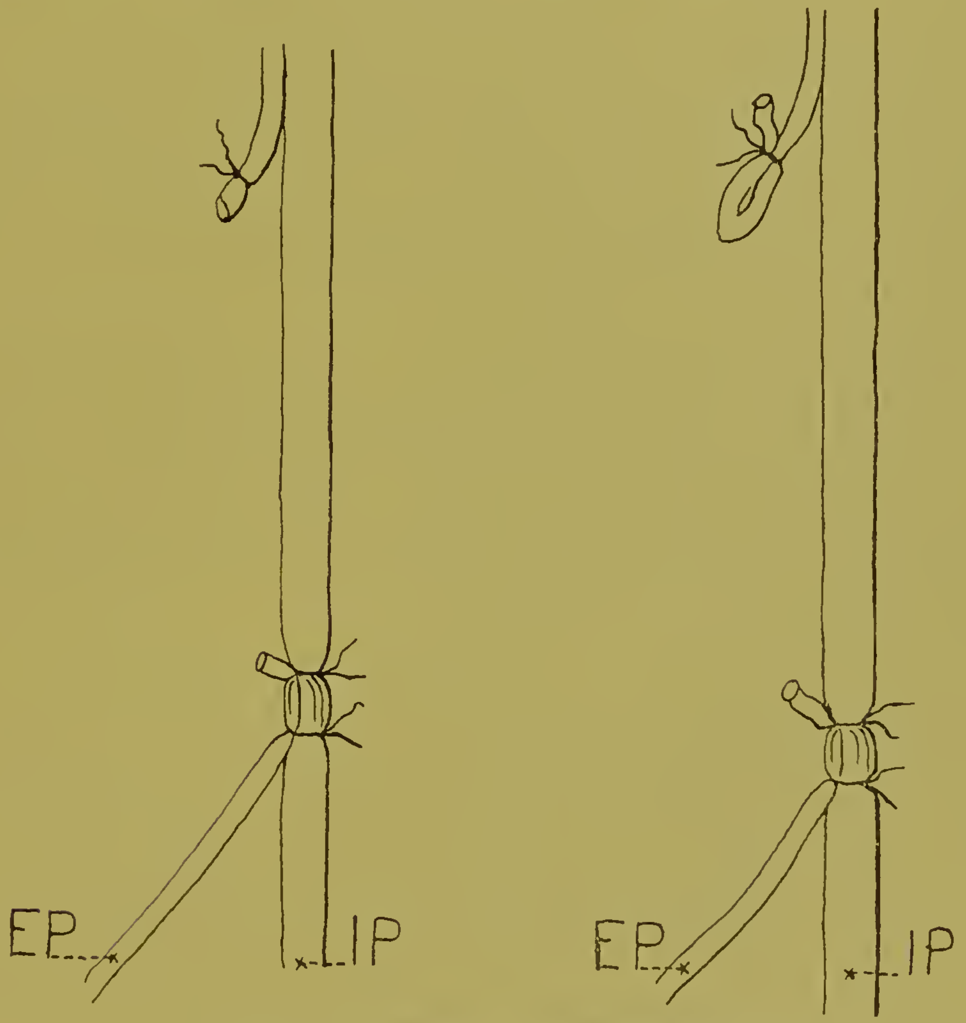
Another explanation might be that the fibres below the neuroma were "embryonic," and that as such, being deficient in the quantity of fatty constituents, they might not give the Marchi reaction like normal nerves. Such an explanation seems as weak as the previous one. In the first place, I do not believe that fibres as old as these (5–6 months) could come under the heading of embryonic fibres; Weigert sections in one of these cases and sections of nerves of the same age and developed under similar conditions in other cases, showed considerable myelin. In the next place, there was in Exp. 120 a moderate Marchi reaction in the corresponding nerve segments, and the new fibres in that case were even younger than in either of the other two, where the reaction was so slight.

Finally, it is to be conceived that under certain conditions the fusion scar might have offered a moderate resistance to the ordinary process of Wallerian degeneration as normally induced by the interruption of a nerve. This resistance might have been due to either chemical or physical causes, or to both. It is manifest that further experiments along this line are necessary not only to explain the phenomena described above, but because they might throw a new light on some of the ordinary questions coming up in the study of nerve degeneration and regeneration.

As regards the clinical aspect of the animals after the various procedures, primary and secondary, no conclusions whatever can be drawn on account of the variability in the paralysis in different cases. In Exp. 120, after the secondary excision of the right EP above the neuroma, the animal's paw did not turn under. But in this case the failure of the paw to turn could not in itself be regarded as evidence for the successful crossing of nerves as resulting from the fusion, because the paw seemed just as normal on the other leg, where a similar excision, but no primary fusion, had been done.

## III. NERVE TRANSPLANTATION BY FUSION.

Fifteen experiments of this class were performed. Six of these are to be regarded as preliminary. In two of the remainder the dogs died on the table while the final physiological tests were being applied. The other seven furnished most of the data on which this portion of the report is based. All the material was examined anatomically.



FIGS. 12 and 13.—Diagrams to show methods of grafting.

Procedure.—After exposure of the sciatic and separation thereof into its two popliteal divisions for a considerable distance by blunt dissection, a piece of the EP was excised, the length varying in different cases from ½ inch to 3 inches. The central stump of the EP was tied off, or looped back, or sewed into the muscle, and the peripheral end was fused to the IP by tying the two together with two or three catgut ligatures, about $\frac{1}{8}$ inch apart (figs. 12 and 13). The highest ligature was placed just below the stump end of the grafted EP. As a rule the united nerves were crushed with a hæmostat in addition. The place of fusion was somewhat below the original bifurcation.

In some cases, as a control, a piece of the EP was excised on the other leg, but of course no fusion was done. The excised piece was of about the same length as that on the other side, and both stump ends were tied off.

Physiological Data.—After sufficient time had elapsed, stimulation of all nerves was carried out before and after division, as described above. The location of the fusion was evidenced by an elongated swelling (neuroma), which was often very slight. The old central stump of the EP could be identified as a small bulb which seemed to end with sufficient abruptness to suggest that no direct connection had been restored between it and its transplanted peripheral stump, although a delicate strip of tissue occasionally extended down from it along the IP. (This contained no nerve fibres. See below.)

A routine order of testing was used in six cases, dividing, 1st, the IP below the neuroma; 2nd, the sciatic high up, just above the old central EP stump; and 3rd, the EP below the neuroma. (The specimen was then removed, mounted on cardboard, and placed in the fixative.) In the seventh case the reverse order of division was used. After each division all stumps connected with the neuroma were stimulated. The responses of the paw and ankle evoked by stimulation in the seven cases are summarised in the accompanying table.

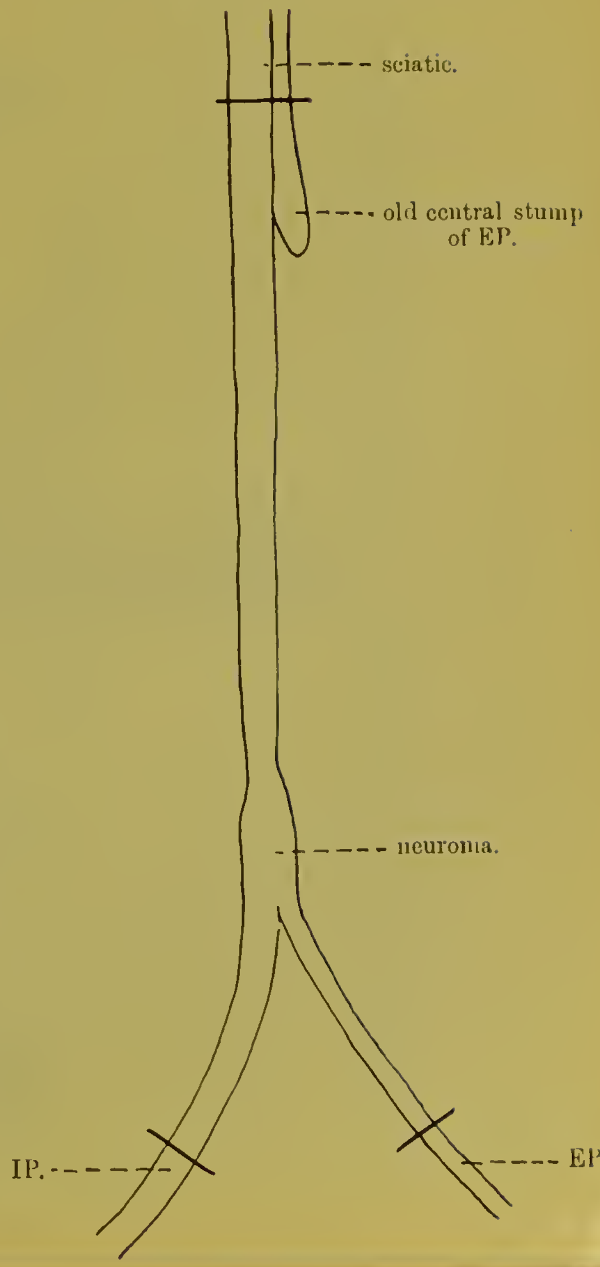
Glancing at this table, it is seen that before division all the nerves responded normally except the IP above the neuroma, which in three cases occasionally responded by dorsal flexion, in addition to its normal response of plantar flexion. After division of the IP below the neuroma, responses evoked by stimulation of its peripheral stump were invariably normal. Stimulation of its central stump showed passage of impulses through the scar and upward as indicated by the increased rate of respiration in two cases, and some reflex skeletal movements in a third case. The response of dorsal flexion of the paw evoked by faradic stimulation of the central IP stump was in no case confirmed by a similar effect with mechanical stimulation, and might possibly have been either reflex or due to escape, although in several cases (this point omitted in the table) the minimal stimulus necessary to evoke response was the same all along the stump, thus ruling out escape. After division of the nerve above the neuroma, mechanical stimulation of the stump above mentioned (stump attached to neuroma below) in two cases (Exps. 114 and 119) evoked responses in muscles supplied by the other nerve suggesting axone reflexes. But if these were axone reflexes, it is necessary to explain why they should not

TABLE TO SHOW RESPONSES OF PAW AND ANKLE EVOKED BY MECHANICAL AND FARADICAL STIMULATION, BEFORE AND AFTER DIVISION.

| No. of experiment. | No. of days after fusion. | Before division. | | | After division of IP below neuroma. | | | | After division of sciatic * above neuroma. | | | | After division of EP below neuroma. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | IP above neuroma. | IP below neuroma. | EP below neuroma. | Central stump of IP. | | Peripheral stump of IP. | | Central stump of IP below neuroma. | | Peripheral stump of sciatic above neuroma. | | Peripheral stump of EP. | |
| | | Farad. | Farad. | Farad. | Farad. | Mech. | Farad. | Mech. | Farad. | Mech. | Farad. | Mech. | Farad. | Mech. |
| 112 | 201 | Plant. flex. | Plant. flex. | Dorsal flex. | Dorsal flex. | Reflex† respr. No paw movement. | Plant. flex. | Plant. flex. | Dorsal flex. | No response. | Dorsal flex. | No response. | Dorsal flex. | Dorsal flex. |
| 114 | 165 | Dorsal and plant. flex. | Plant. flex. | Dorsal flex. | Dorsal flex. reflex skeletal movements. | No note. | Plant. flex. | No note. | No note. | Dorsal flex. | Dorsal flex. | No note. | Dorsal flex. | No note. |
| 118 | 161 | Plant. flex. as a rule. | Plant. flex. | Dorsal flex. | Dorsal flex. | No response. | Plant. flex. | Plant. flex. | Dorsal flex. | No response. | Dorsal flex. | Dorsal flex. | Dorsal flex. | Dorsal flex. |
| 121 | 150 | Plant. flex. as a rule. | Plant. flex. | Dorsal flex. | Dorsal flex. | Reflex respr. | Plant. flex. | Plant. flex. | Dorsal flex. | No note. | Dorsal flex. | Dorsal flex. | Dorsal flex. | No note. |
| 122 | 127 | Plant. flex. | Plant. flex. | Dorsal flex. | Dorsal flex. | No note. | Plant. flex. | Plant. flex. | Dorsal flex. | No note. | Dorsal flex. | No response. | Dorsal flex. | Dorsal flex. |
| 123 | 127 | Plant. flex. | Plant. flex. | Dorsal flex. | Dorsal flex. | No response. | Plant. flex. | Plant. flex. | Dorsal flex. | No note. | Dorsal flex. | No response. | Dorsal flex. | Dorsal flex. |
| | | | | | After division of EP below neuroma. | | | | After division of sciatic above neuroma. | | | | After division of IP below neuroma. | |
| | | | | | Central stump of EP. | | Peripheral stump of EP. | | Central stump of EP below neuroma. | | Peripheral stump of sciatic above neuroma. | | Peripheral stump of IP. | |
| | | | | | Farad. | Mech. | Farad. | Mech. | Farad. | Mech. | Farad. | Mech. | Farad. | Mech. |
| 119 | 163 | Plant. flex. | Plant. flex. | Dorsal flex. | Plant. flex. | Reflex respr. No paw movement. | Dorsal flex. | Dorsal flex. | Plant. flex. | Plant. flex. | Plant. flex. | Plant. flex. | Plant. flex. | Plant. flex. |

* Sciatic, strictly speaking, but practically only the IP, as most of the EP had been excised above the neuroma.

DIAGRAM TO SHOW PLACES OF DIVISION IN STIMULATION TESTS.



have been obtained by mechanical stimulation before the upper nerves were divided. The most plausible explanation is that these reflexes were inhibited so long as the connection with the central nervous system was preserved.

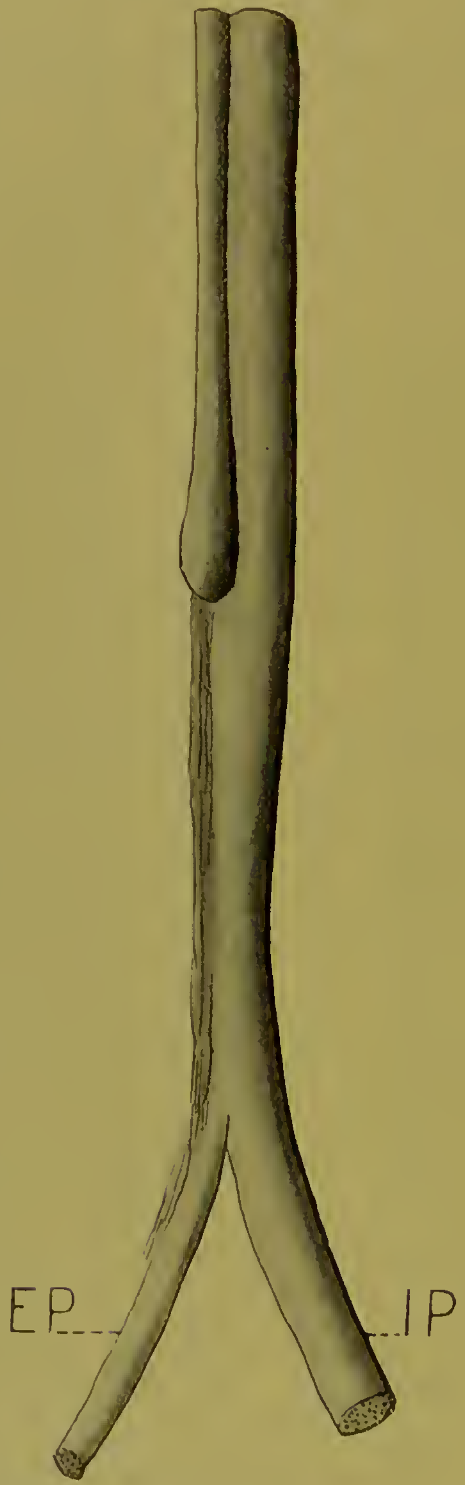


Fig. 14.—Exp. 107. Enlarged representation of graft 4 months after operation. The upper stump of the EP has formed into an elongated bulb.

After division of the EP below the neuroma, stimulation of the peripheral stump evoked normal responses.

Summarised, the data in all cases showed conduction of impulses from the main IP trunk downward through the scar, both along their original course and into the transplanted EP; and in some of the cases, in addition, conduction upward through the scar. In at least two cases there was also evidence of the passage of impulses from the lower segment of one nerve through the scar and into the lower segment of the other (axone reflex).

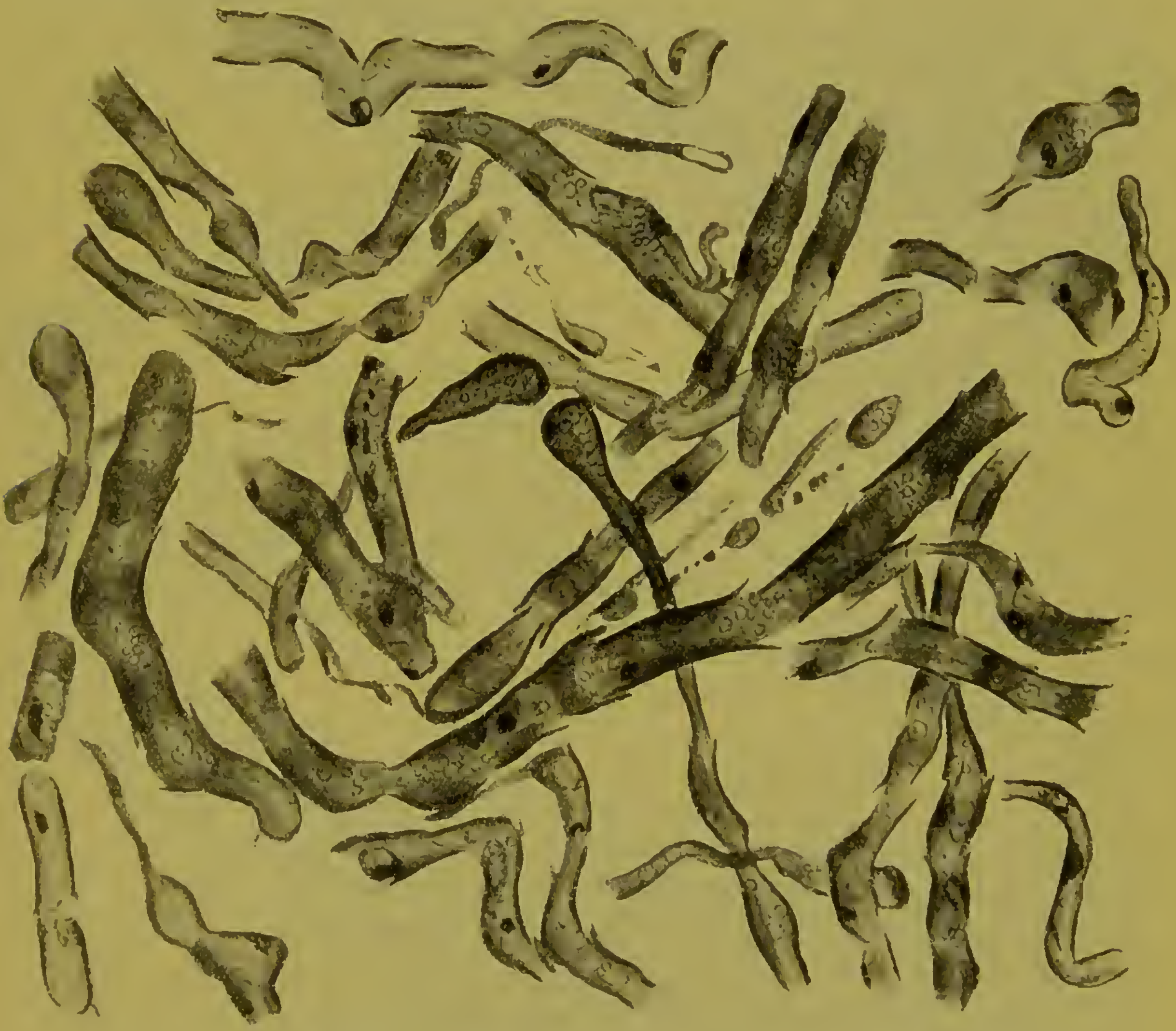


FIG. 15.—Exp. 114. Regenerating nerves crossing in scar. Autopsy 165 days after fusion. (Drawn under oil immersion, $\frac{1}{12}$ objective, 4-eyepiece, Zeiss. Weigert-Pal stain.)

Where a piece had been excised from the EP on the other (control) leg, its peripheral stump invariably failed to respond.

Anatomical Data.—The gross specimen sometimes, as above mentioned, showed a marked neuroma at the place of fusion. In most cases, however, the nerves seemed to spring from one trunk with but the slightest swelling. This appearance (fig. 14) was in striking contrast to the normal, for, normally of course, the two popliteals remain separate in a common sheath even after joining to form the so-called sciatic. Just above the region of

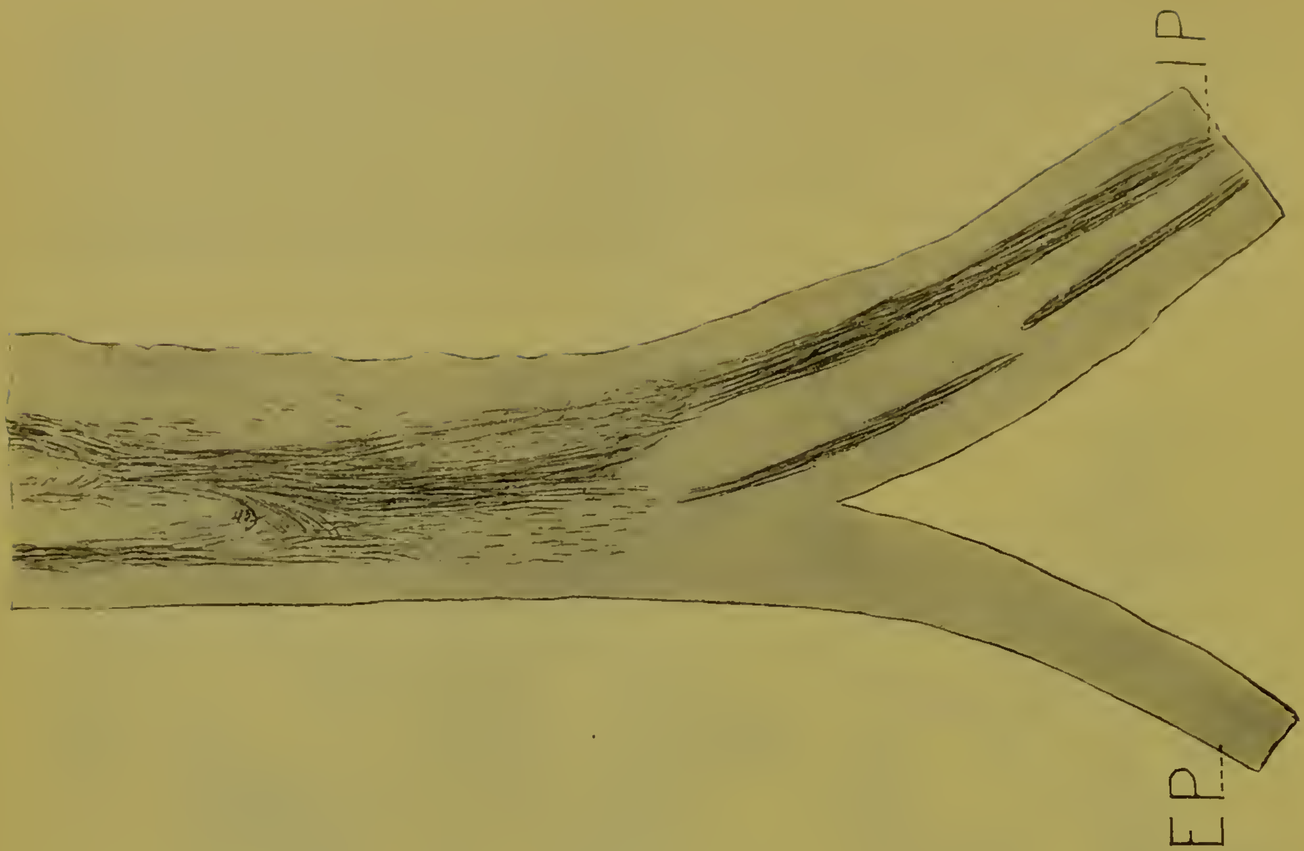


Fig. 16.

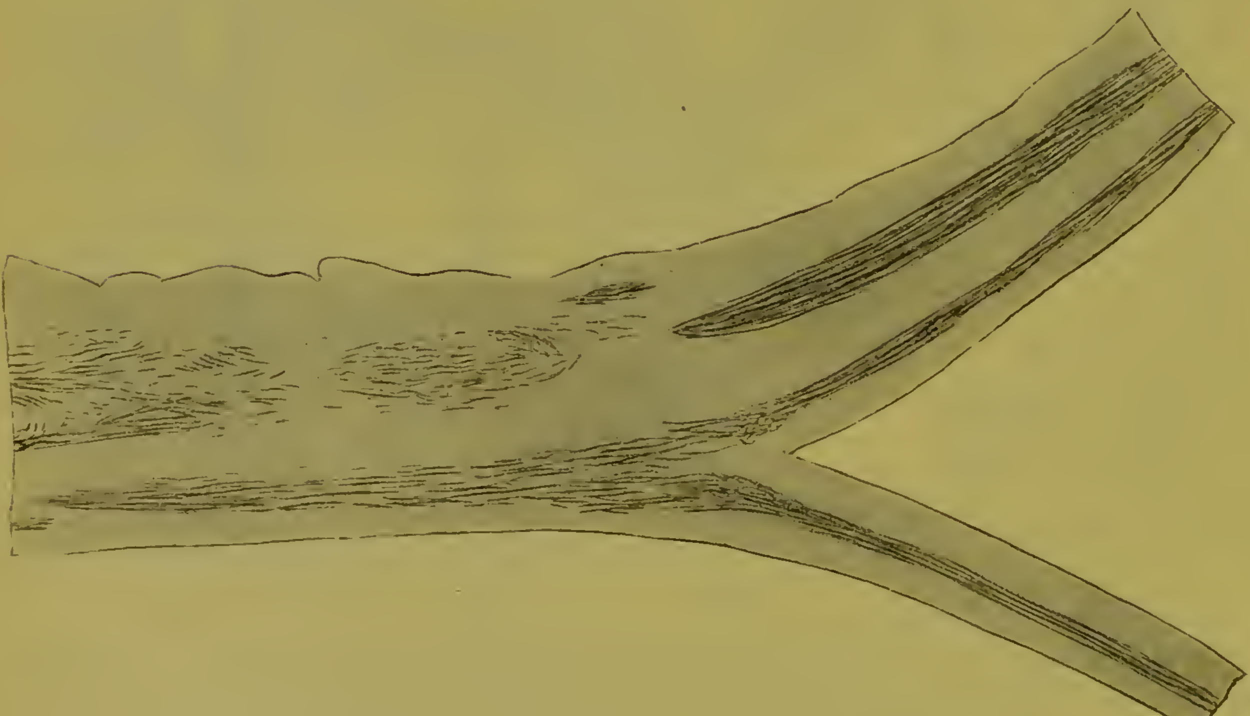


Fig. 17.

Figs. 16, 17, and 18.—Exp. 119. Semidiagrammatic representations of longitudinal sections through region of grafting. The three sections are selected from the same series, and in that series are numbers 33, 67, and 110 respectively. Autopsy 163 days after fusion. (Drawn with help of dissecting microscope. Enlargement, 8 diam. Weigert-Pal stain.)

fusion the IP was occasionally constricted for a short space. The nerves below the bifurcation had the colour and size of normal nerves.

Histological Summary.—Sections through neuromata showed about the same conditions as corresponding sections obtained by fusion in continuity and earlier described, the important point being that the new fibres regenerating in the scar were laid down in all directions as necessitated by the proliferated mass of cells that formed immediately after the fusion. The result was frequent crossing of fibres (fig. 15), free passage of fibres into both the original and the grafted nerves (figs. 16, 17, 18), and in several cases an actual neural connection in the scar between the lower segments. The other findings, such as fibrillations and end brushes, spirals, and so on, are those always accompanying nerve regeneration after interruption. The Cajal method showed these characteristics best.

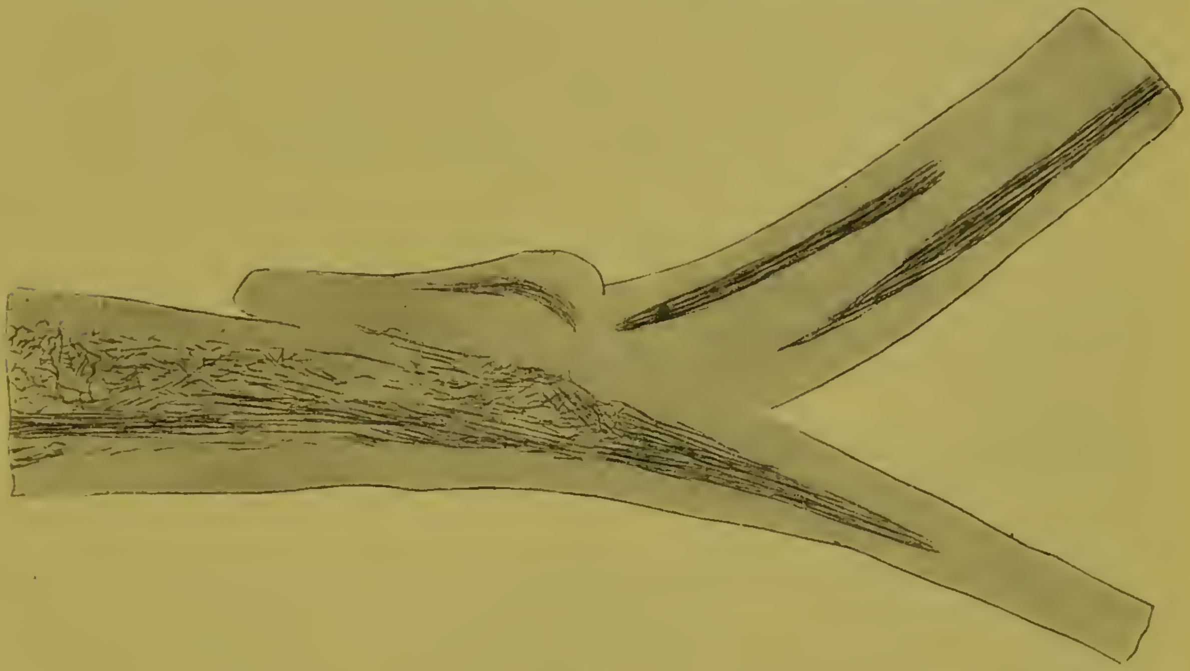


Fig. 18.

Below the neuromata both nerves showed regeneration fairly advanced by the end of three months after the fusion. The fibres were, however, smaller than normal, and the interspaces larger. As to this interspacing, it is perhaps important to mention that the fibres were distributed rather uniformly throughout the cross-section. Regeneration was equally advanced all along the course of the nerves, but in several cases it was further along (as evidenced by the size of the fibres) in the IP than in the EP.

The delicate tissue sometimes seen to pass down from the old EP stump was found to contain no fibres.

On the control side the peripheral stump of the EP showed no sign of fibre formation.

Nerve-Counts.—These were made in three cases with the following results:—

| Upper set. | | | Lower set. | | | Increase. |
|---|---|---|---|---|---|---|
| Dog 112 | IP | 11,468 | EP<br>IP | 3399<br>9196 | Total<br>12,595 | 1127 |
| Dog 114 | IP | 10,342 | EP<br>IP | 4246<br>6790 | Total<br>11,036 | 694 |
| Dog 118 | IP | 11,165 | EP<br>IP | 3152<br>9056 | Total<br>12,208 | 1043 |

This table seems to show that there was a decrease in the number of fibres contained in the IP below the neuroma, but the two lower nerves together contained a greater number than the IP trunk above the neuroma. For a reason cited earlier, the apparent increase did not necessarily prove branching in the scar. Be that as it may, it was plain that regeneration took place in about as many sheaths as contained fibres in the first place.

## IV. General Conclusion.

On a basis of all the experiments cited, it seems reasonably safe to state that if two nerves are joined together by either of the procedures above described, there usually takes place, 1st, regeneration and restoration of conductivity in both nerves below the scar; 2nd, conduction of impulses through the scar, both along the original paths and from one nerve to the other; and 3rd, crossing of fibres within the scar, as shown anatomically.

## V. Relation of Fusion Method to other Methods of Nerve-crossing.

Previous researches in nerve-crossing have been carried out by suture methods. Among the more important of recent researches are those of Kilvington and Osborne (8). Comparing their findings with my own, I find considerable similarity, although these authors placed more reliance on the results obtained by faradic stimulation than I do. It is interesting to compare Kilvington's (7) nerve-counts with mine. In two of his three cases the EP was the upper nerve used. His results were:—

| Upper set. | | | Lower set. | | Total. |
|---|---|---|---|---|---|
| Dog II. | EP | 4435 | EP<br>IP | 2786<br>7196 | 9982 |
| Dog III. | IP* | 7740 | EP<br>IP | 2431<br>7275 | 9706 |
| Dog IV. | EP | 5826 | EP<br>IP | 1880<br>6808 | 8688 |

* Not completely divided.

My total is always larger, but I dealt with more fibres in the upper set, so that relatively Kilvington's results show a greater increase. It will be interesting at some future time to graft the IP into the EP by the fusion method in order to obtain a more accurate comparison.

As regards the laws for the union of nerves which are set forth by Langley and Anderson (13), and which are also based on suturing experiments, I am as yet in no position to state whether or not the results obtained by fusion conform to these laws. But I hope to have the opportunity of repeating by the present method some of the experiments done by these authors.

I have, however, examined a number of scars resulting from suture operations, and from these data and the data described by other investigators, I feel convinced that the results obtained by suture are nothing more nor less than the effects of conditions superinduced by scar; for no matter how skilfully suturing is carried out, the final arrangement of the new nerve fibres must depend on the mechanical conditions necessitated by the mass of proliferated cells which forms at the divided nerve ends. This mass of cells prevents the cut ends of the nerves from remaining in accurate apposition so as to enable fibres to pass directly from one fascicle into another. From this view-point the two methods are the same.

The advantages of the fusion method are, however, in its physical possibilities. The method enables the physiologist to carry out procedures not to be thought of by the other method. The union of nerves in their continuity, the anastomosing of nerves of most diminutive size, and experiments in distortion of nerve pattern of single fascicles, are all feats of technical simplicity, if this method is employed.

In conclusion, I wish to acknowledge my indebtedness to Professor George N. Stewart, Director of the Laboratory, for valuable help all through the research, and important suggestions in the preparation of the manuscript. I am also much indebted to Dr O. T. Schultz for the microphotographs.

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# THE EFFECT OF NERVE-FUSION ON THE PATTERNS OF NERVES CONTAINING DEGENERATED FIBRES. By HENRY O. FEISS. (From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University.) (With five illustrations in the text.)

(*Received for publication 9th July* 1912.)

NERVE anastomosis has for its aim the guidance of nerve fibres into new paths, so as to bring about alteration of function, or, in cases of degeneration, restoration of function. Anatomically speaking, such an aim can be fulfilled only by a change in nerve pattern. A review of the more important investigations on this subject, a few years since, by Kilvington (1) showed a steadily increasing knowledge as regards the theoretical possibilities of the procedure, and this author, in collaboration with Osborne (2), has in more recent years reported some interesting experiments showing the effects of nerve anastomosis on nerve patterns, with special reference to the factor of fortuitous apposition of fibres in the united segments, and to the question of bifurcation of axones. As regards the kinds of nerves which may be joined with functional results, Langley and Anderson (3) have investigated this aspect and have been able to come to rather definite conclusions.

Leaving clinical reports of human subjects out of consideration, because anatomical control is, in such cases, practically out of the question, one finds that in almost all the previous researches on animals the nerves dealt with have been normal ones. Kennedy is, I believe, the only investigator who has brought about primary degenerations and later grafted good nerves into the nerves thus degenerated. He reports four cases (4), one monkey and three dogs. In the monkey he cut the facial nerve, and one month later he joined the spinal accessory to it. He reports restoration of function in the eye in two months. In the three dogs he also cut the facial. In two of these he waited one month, and then grafted into it the spinal accessory in the one case and the hypoglossal in the other. In the former there was no recovery of function at the end of sixty-nine days, when the animal died, and in the other voluntary dissociated closure of the eye returned. In the third dog he waited one hundred days after cutting the facial, when he joined to it the spinal accessory and obtained a return of eye-closure in one hundred and sixty-seven days. These anastomoses were all done by end-to-end suturing. In his seventh conclusion of this same report he states that the only way to make an efficient union between two nerves is to completely cut across all the nerve fibres in both nerves. According to him, therefore, it is plain that in order to achieve successful restoration of function in one nerve it is necessary to sacrifice another.

In these experiments of Kennedy the primary lesions were in the peripheral nerves themselves. No previous investigator had made it a point to ascertain the effects of nerve-crossing in animals, where degeneration had resulted from lesions produced primarily some time previously, in or near the central nervous system. From a surgical point of view the palsies of infantile paralysis are here concerned. From a physiological point of view the significance of the problem is too apparent to require comment.

This is one gap in the history of the investigation which I have endeavoured to study, and in a previous report (5) in connection with another subject I have already presented some of my experiments that pertain to this point. The plan that I usually followed was to produce lesions in the lumbar spinal cord of dogs. Marked paralyses were thus achieved, and afterwards these paralyses were systematically and repeatedly studied, so that there was no question as to their permanence. An attempt was then made to distort the nerve patterns in the nerves containing most degenerate fibres, these operations being carried out in the sciatic nerves or in the anterior roots.

The method that is commonly employed to anastomose nerves is to combine them by suture, either end-to-end as exemplified by Kennedy's experiments, or by cutting one nerve and sewing it into a gap made in another. In my work, however, I resorted to a different procedure. The reasons for my departure from the older methods were briefly mentioned in a preliminary report (6). In the present connection it is expedient to discuss some of these reasons more fully.

In the first place, as regards the nerve patterns of peripheral nerves which are derived from portions of the cord containing a lesion: such nerve patterns would, of course, vary greatly in different cases, according to the extent and location of the lesion, but even so, the degenerated areas in the cross-sections of the peripheral nerves would not show a definite grouping to conform with the cord lesion, no matter how definitely circumscribed the latter may be. A cross-section of such a nerve usually shows degenerated sheaths scattered irregularly in various parts. Rarely does one find an entire fascicle gone. This of course is due to the reassortment of fibres which occurs in the plexus. Owing to this a nerve trunk is made to consist of all sorts of fibres, motor, sensory, and sympathetic, gathered from rootlets at many levels, and even if it is supposed that a given set of fibres is represented by a given nucleus in the cord, it could only be by the rarest chance that such a nucleus would be selected by a lesion without damage to many others, owing to their great overlapping. In my experiments I deliberately tried to confine the lesion to a limited region of the cord, and so by injuring a sufficient amount managed in some cases to include enough nuclei and roots to cause motor degeneration of whole nerves. But in infantile paralysis the pathological lesions themselves are irregular and scattered, so that it is far rarer for a single nerve-branch to be completely degenerated than for many branches to contain degenerated fibres scattered here and there among the good ones.

Under these conditions one could not graft good branches into bad ones, because such branches do not exist as such.

On the other hand, if one based the anastomosis on the result of electrical stimulation, one might fall into the error of mistaking an almost completely degenerated branch for a good one, because a very few good motor fibres in a bundle are sufficient to ensure positive responses if stimulated. Moreover, even where it is possible to sort good fascicles from bad ones, the procedure of dividing nerves and suturing them to others seems a waste of good nerve substance, because in severing a good nerve from one peripheral connection and grafting it to another, a new and permanent loss of function is entailed, perhaps as important as the original paralysis.

Finally, to attempt to establish connections in the hope of conveying fibres directly from one nerve into another by suturing them together, or, in fact, by any method whatsoever, is to attempt the impossible. Anything in the nature of a trauma to a nerve calls forth a marked tissue reaction, due partly to ordinary pathological changes occurring in the connective tissue constituting the outer sheaths, and more particularly owing to the peculiar proliferative process of the neurilemma sheath cells. Moreover, the presence of the suture material, which acts as a foreign body, increases the tendency to tissue reaction in a marked degree. As a result, a neuromatous mass of new connective tissue, containing leucocytes, vessels, and proliferating sheath cells, necessarily forms and comes to intervene between the cut surfaces of nerves thus sutured together, no matter how accurately the operation is done. It is safe to state that no fibres growing from the central stump of one nerve can be guided directly into another. They must one and all enter and pass through the mass of new tissue which forms between the divided nerve ends. So if suturing is resorted to, the operator must face this anatomical fact and deal with it as an unavoidable condition.

For these reasons I gave up the method of grafting nerves by division and suture and substituted that of "nerve-fusion." This procedure consists simply in tying absorbable ligatures about the nerve or nerves the patterns of which are to be distorted. The ligatures must be tied tightly enough to crush the outer sheaths. The procedure has for its chief aim the utilisation of the peculiar properties of the interposed scar in neurotisation, at the same time ensuring at least a theoretical chance for a new central connection for every sheath in the peripheral segments.

If two normal nerves, such as the internal and external popliteals, are combined by this procedure, the effects are those previously described in this

Journal (7). Briefly summarised, they consist of a marked cellular proliferation at the location of fusion, thus constituting a neuroma. If the perineuria of the nerves are sufficiently traumatised, the proliferating cells in the scar show no signs of the parallel arrangement of the nerve sheaths above, but they run in all directions. This is significant, because, being laid down before the regenerating fibres, the latter must assume directions necessitated by the arrangement of these cells. The result is that the new fibres derived from the central nerve segments run in all directions and cross each other frequently. There is also evidence that many of the fibres form branches.

The essential fact derived from these experiments is that the scar tissue or neuroma is dealt with as a means of distorting the nerve pattern, because every fibre from the central segment has to pass through it, and in passing through becomes subject to the mechanical conditions of the cells of which it is made up. On account of the indiscriminate arrangement of these cells, the influences for reneurotisation are no greater in one part of the scar than in any other; therefore, at a certain stage after the operation, each square millimetre of the cross-section presents about an equal amount of newly formed nerve substance. The same is usually true in the nerve fascicles of the peripheral nerve segment. Such fascicles show a reasonable uniformity in the amount of regeneration of all the fibres cut in a given cross-section.

This brings us to the consideration of the chief problem of the present inquiry, namely, the effect of interposing such a neuroma in the course of nerves which are abnormal. Does the uniformity of reneurotisation, which occurs where normal nerves are fused, apply to nerves some of the fibres of which have been degenerated by primary lesions? Will such a neuromatous mass, by thoroughly mixing the pattern, as in the normal, serve to supply all the sheaths in the peripheral segments, both good and bad, with the means of deriving a new and equal neurotisation?

To answer this question, I will first describe in some detail an experiment not heretofore reported, and which is most suggestive in illustrating the effects of the procedure. The case is one in which two important anterior spinal nerve roots had been excised in order to bring about the initial degeneration. Following is the case:—

### EXPERIMENT 63.—FULL-GROWN, MALE, BULL TERRIER.

Jan. 13th, 1910.—Under complete ether anæsthesia, laminectomy of 5th and 6th lumbar vertebral arches performed. Two large anterior roots on right side seized and excised. At the time it was not certain which roots these were. Muscle, fasciæ, and skin were closed in one layer.

For the next ten days the animal showed marked weakness of both hind legs and failed to move tail. Some swelling about the wound.

On Jan. 24 swelling had gone down and both hind legs were flexed and slightly crossed and he seemed to have but little power in either of them. From this date onwards he improved.

March 15 (61 days). Status:—

Attitude.—Stands on three legs, not resting on right hind leg. Hip acutely flexed, knee flexed to a right angle. Ankle extended, and foot and paw hanging loosely. Left hind leg held naturally.

Gait.—Runs on three legs, occasionally bringing right hind leg to the ground, but bearing no weight on it. Cannot place paw on planta, as it turns over.

Active movements.—Occasionally moves hip and knee of affected leg. Seems to make no active movements in the two lower joints. Tail seems under control.

Passive movements.—Flexion of knee and hip carries with it no flexion of ankle and paw. Extension of knee and hip accompanied by extension of ankle and paw towards end of movement. Movements of both knee and hip very free, suggesting weakness of quadriceps. Passive resistance in movement of ankle about equal on both sides.

Response to stimulation.—He seemed to feel the prick of a pin poorly on the right leg, below the knee.

A test-tube filled with hot water was felt all over the leg.

Temperature between the toes.—Right, 28·5°. Left, 28·5°.

Knee-jerks.—Both present, but not as lively on the right as on the left side.

Anal reflex present.

Sphincter control present.

Response to faradic current applied externally. Sluggish below knee of right leg on anterior and external aspects.

Remarks.—Distinct atrophy of muscles of right leg.

March 19 (65 days).—Under complete ether anæsthesia, the sciatic nerve of the right leg exposed. The popliteal nerves stimulated with the faradic current. No response evoked in the external popliteal, but on stimulation of the internal popliteal the paw and ankle responded normally with plantar flexion.

The popliteals were crushed and squeezed together at the bifurcation and two catgut ligatures applied $\frac{1}{4}$ inch apart.

After this operation no change was noted till June 12, when the animal seemed to show a slight improvement in the gait.

Nov. 27 (253 days after fusion).—Status about the same as on March 15, except for slight improvement in gait and for the fact that the knee-jerks seemed equal in the legs.

On this date (Nov. 27) the sciatic was again exposed under complete ether anæsthesia. A neuroma one inch long indicated the region of fusion. Stimulation of the external popliteal now evoked dorsal flexion of the paw and ankle and of the internal popliteal plantar flexion as before Both nerves responded after division as well.

The animal was sacrificed, and the specimen of the neuroma with the popliteals and the sciatic were placed in Müller's fluid.

Dissection of the cord showed 5th and 6th right anterior roots missing. The dog had only twelve ribs.

## ANATOMICAL REPORT OF NERVE SPECIMEN.

Sections taken from sciatic above the neuroma, from the neuroma, and from the popliteals below. These stained by Weigert-Pal and general stains.

Sections from above the neuroma show numerous sclerosed tracts resulting from the original root excision. These sclerosed tracts are for the most part scattered over various parts of the cross-section (fig. 2).

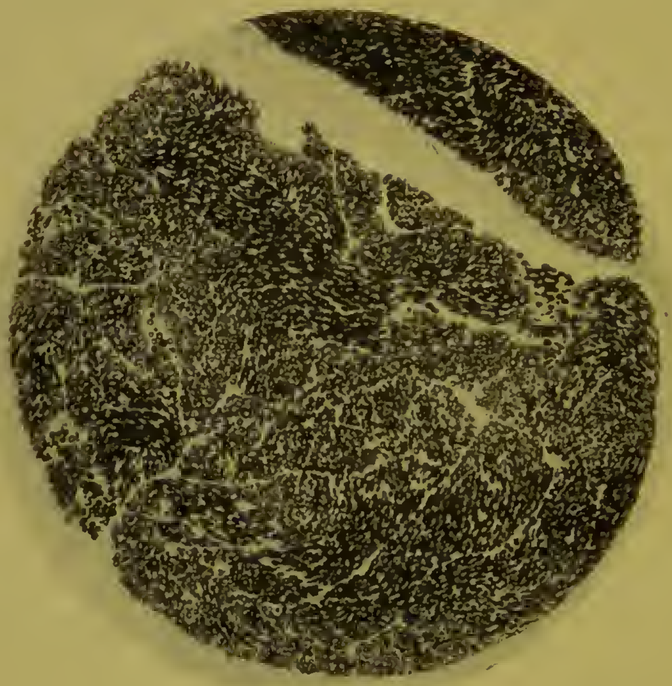

FIG. 1.—Exper. 63. Section through normal internal popliteal nerve (left).
All the microphotographs are from Weigert-Pal preparations and are enlarged 45 diameters.

Sections from the neuroma show nerve fascicles enlarged and misshapen in outline. The epineurium often absent, or rather blended with new connective tissue of which there is a large amount both within and without the fascicles, the two being directly continuous in many places. All this new tissue contains many nuclei. Newly formed small nerve fibres are to be seen everywhere. They lie between the cells and extend in all directions. Although the majority are still within the original epineurium, a large number have broken through and are scattered throughout the sections.

Sections from below the neuroma, through the popliteal nerves, show fairly well-regenerated fibres, singly and in groups, with a large amount of connective tissue like the sclerosed tracts above. These represent empty sheaths. It is very plain that regeneration is not uniform in the cross-sections.

Nerve-counts.—These were made by an expert microscopist[1] using a high power. Only fibres contained in the internal popliteal were counted.

Count according to fascicles:—

| | |
|---|---|
| 4 fascicles above neuroma | 51 |
| | 634 |
| | 1,514 |
| | 11,991 |
| Total | 14,190 |
| 7 fascicles below neuroma | 8,246 |
| | 372 |
| | 339 |
| | 430 |
| | 442 |
| | 1,096 |
| | 1,487 |
| Total | 12,412 |

As regards the other cases pertaining to this aspect of the investigation, it is not desirable to report them in full in the present connection, as most of the data have been summarised in the report alluded to above (5). In all of these the primary paralyses were obtained by direct lesions in the lumbar cord. In two of this group the paralysis was confined chiefly to the tail, and for this reason a fusion of the lower cauda was later carried out. The anatomical and physiological data were very difficult of interpretation, but functionally in both cases the tail seemed to be under better control a few months after the fusion.

In six of the other experiments the primary lesions in the cord brought about distinct leg palsies of various kinds. In three of these cases a distortion of nerve pattern was attempted by fusing the anterior roots on one side. The results were either negative or increased paralysis. In the three others the sciatic was attacked. Two of these failed of completion. In the third, Exper. 64, the residual paralysis, some months after the lesion, was very similar to that of the case here reported (Exper. 63). The fusion was done ninety-nine days after the primary lesion. One hundred and ninety-three days later the nerves were investigated. Anatomically the appearance of sections above and below the fusion-neuroma showed a condition very similar to that of corresponding sections in Exper. 63. Degenerated sheaths were scattered throughout the sections showing little if any apparent effect from the fusion. Functionally and physiologically no alteration was observed.

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[1] I am indebted to Mr Richard Muir of the Pathological Department of the University of Edinburgh both for the nerve-counts and for the microphotographs.

These two cases (Expers. 63 and 64) may be regarded as typical of this class of experiments. In the one, the primary palsy was produced by excising two important roots; in the other, practically the same picture happened to be produced by direct traumatisation of the cord. In both, the muscles supplied by the external popliteal were markedly palsied and a distortion of nerve pattern was attempted by fusing the two popliteals together. In the one case there seemed a slight improvement in function; in the other there was none. In both, the histological sections of the nerves below the neuroma showed that many sheaths had failed in neurotisation, although numbers of fibres in advanced stages of regeneration were evident.

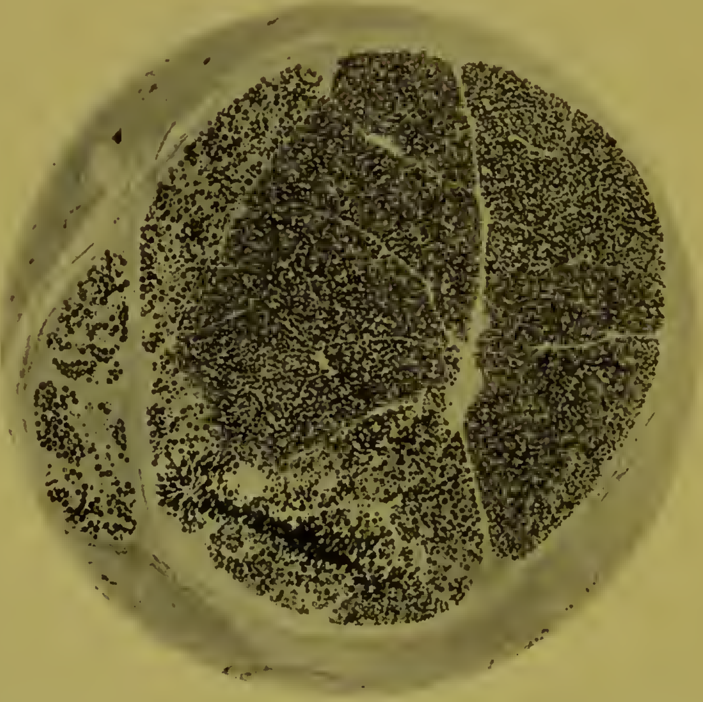

Fig. 2.—Exper. 63. Section through right internal popliteal nerve above fusion neuroma, showing sclerosed tracts resulting from root excision.

It must be borne in mind that, in attempting the distortion of pattern by fusion, every fibre below the scar became degenerated for the time being, which of course implies that such fibre sheaths as had escaped the primary cord or anterior root lesion were, temporarily at least, reduced to the same state as those originally degenerated. This refers not merely to sensory and sympathetic fibres, but also to such motor fibres as entered into the formation of the sciatic by roots above and below the affected ones.

Naturally this was the basis of expectation for more positive results, for if every sheath below the scar was empty and this scar came to contain a mass of regenerating fibres and fibrillæ indiscriminately arranged and of about the same neurotisation value throughout the cross-section, it might well have been expected that, once regeneration had begun in the peripheral segments, neurotisation would take place uniformly in every part of the fascicles, as is usually the case after fusion of normal nerves.

But no such result was obtained. After a sufficiently long interval for regeneration to occur, many empty tracts were still evident, mingled with many fibres that had reached advanced stages of regeneration. It is therefore to be inferred that fibres once degenerated in or near the central nervous system cannot, after a certain interval of time (which in the case of root-severance is within sixty-five days), reap any important advantage from the operation of local nerve distortion by fusion, and, reasoning by analogy, by any other method of nerve-crossing.

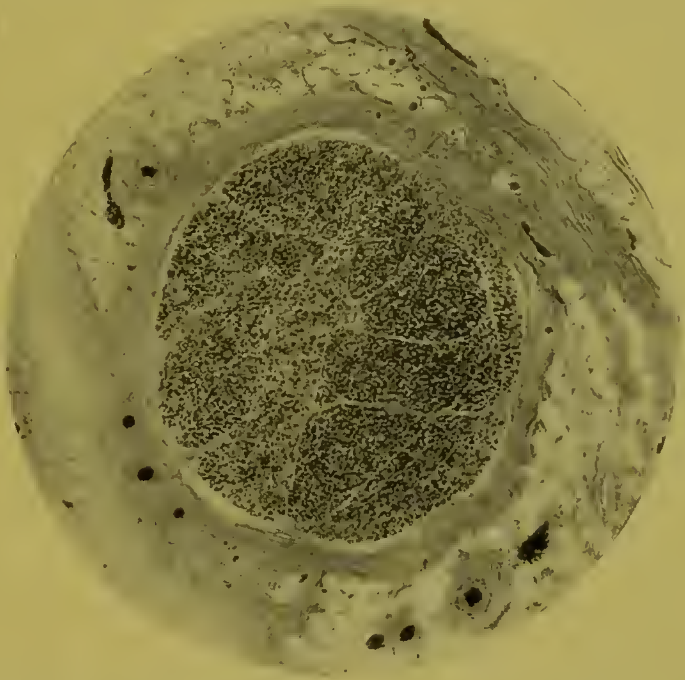

Fig. 3.—Exper. 63. Section through right external popliteal nerve below fusion neuroma. Both this and the next figure show the lack of uniformity of regeneration, with the persistence of empty tracts.

This would go to show that the time-interval between the primary degeneration and the secondary operation of nerve distortion may be of vital import in determining the possibility of restoration of muscular control. Hence in traumatism of the cord or roots, in infantile paralysis, cord tumours and similar conditions, operative interference with the purpose of restoring motor function, if carried out at all, should be done early.

The question arises as to whether the empty tracts evident after the fusion operation were the same as those emptied by the primary operation. To answer this the nerve pattern of sections above the neuroma were (in Exper. 63) compared with sections taken from below the neuroma, but owing to the fact that the nerve had formed into smaller branches, as is, of course, normally the case, corresponding fibres could not be identified.

The answer hinges on the results of the nerve-counts made in Exper. 63, in sections of the internal popliteal nerve above and below the neuroma. These suggest that the effect of the fusion on the nerve pattern of the lower segment was not great. The diminution in the number of fibres was just about what might normally be expected in the more peripherally located segment owing to branches and collaterals, which are constantly being sent off to supply the tissues. It is worth noting that one fascicle above the neuroma contained 1514 fibres, and that one below the neuroma contained 1487 fibres, a diminution of only 27 fibres. It is probable that these represent one and the same collection. What is especially interesting is the fact that there is no increase in number. Therefore, even if it is not completely proven, it is at least strongly suggested that the sheaths originally emptied remained empty after the fusion.

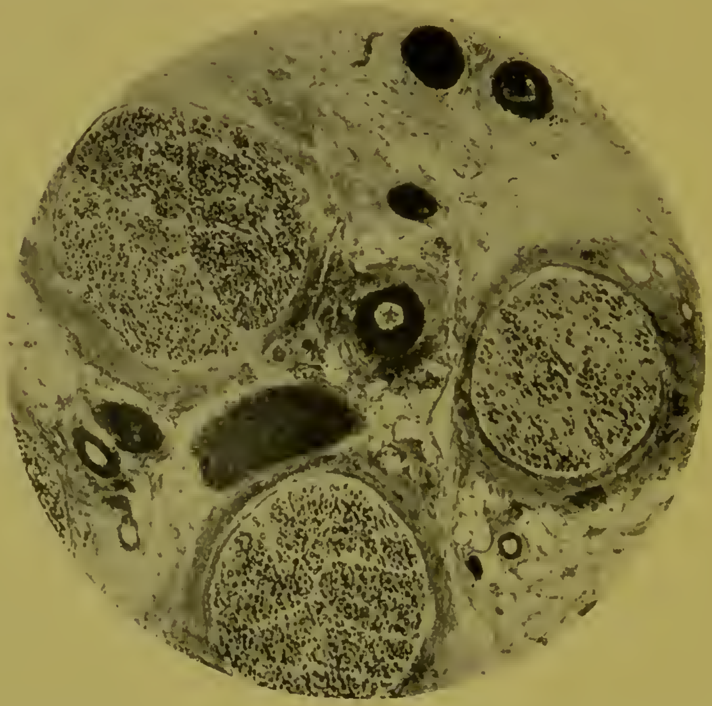

Fig. 4.—Exper. 63. A few fascicles from a section through the internal popliteal below fusion neuroma. Compare with fig. 5.

Of course, even if that is so, it is still very possible that a crossing of the residual fibres may have been occasioned corresponding to the effect of fusion of normal nerves. This was suggested in Exper. 63 by the histological appearances in the scar, and possibly also by the changes in the physiological and functional aspect. In the tail cases there seemed some little improvement in function. If it is assumed that none of the empty sheaths were entered by regenerating fibres, one possible explanation for the changes is that some of the residual fibres had made more advantageous central connections as a result of the fusion. But it is also likely, at least as regards the functional change, that some muscles originally not actively used, and which were supplied by roots above and below the lesion, now assumed vicarious activity by adaptation to new conditions, and that this activity became more pronounced as time went on.

It must not be supposed, however, that the qualitative functional and physiological properties of the fibres themselves could have been affected, for these could only depend on the peripheral end-organs of these fibres. If the distortion produced any changes in these fibres, they could only have been changes in control and adaptability to response brought about by connections with other neurones.

It is safe to suppose that, although a restoration of pattern was not achieved, some distortion was nevertheless probably obtained, but that this pertained only to such fibres as were not affected by the original lesion.

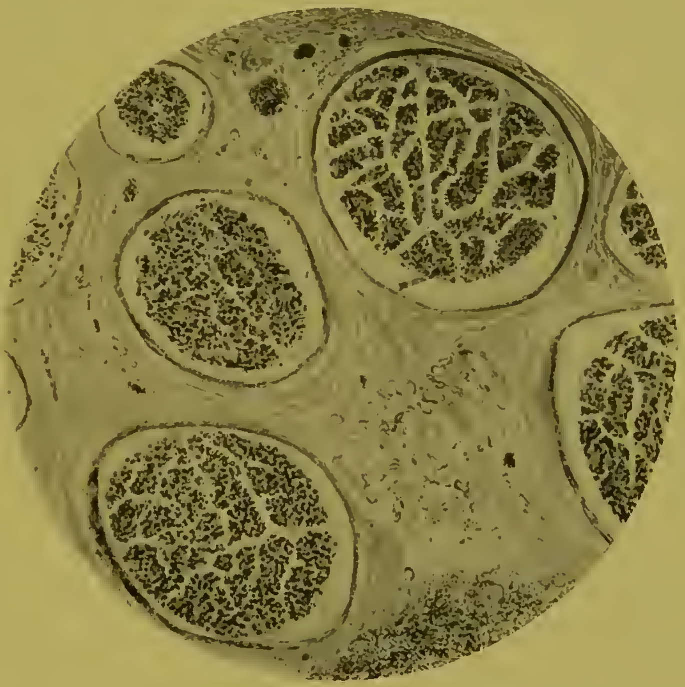

Fig. 5.—Fusion of normal nerves. A few fascicles from a section below fusion neuroma. Note the uniformity of regeneration as compared with that shown in figs. 3 and 4.

A point for consideration is the contrast between Kennedy's rather favourable functional findings and those in the cases here reported, where functional improvement was nil or slight. It was pointed out at the beginning as a reason for this work, that Kennedy did his primary operation in the peripheral nerves. In the above experiments the primary operations were done in or near the central nervous system. Bethe (8) has stated that the closer to the cell of origin a nerve is injured or divided, the less chance is there for restoration. This is undoubtedly true, and explains the poor functional gain in the above cases as well as the differences between the results obtained in Exper. 63 and Exper. 64, the latter being a more central lesion and showing no improvement whatever.

There remains to consider what part chemotaxis took in the second neurotisation. Turning again to Exper. 63, it has already been made evident that only certain sheaths became refilled, and it is likely that these were for the most part the same as escaped degeneration after the original root excision. This fact is consistent with the most plausible view of chemotactic influence, for it is more than likely that the newly degenerated fibres (after fusion) would have a stronger chemotactic power than those that had been degenerated some time (as a result of the original lesion), and so the sheaths originally emptied, though perhaps in other respects subject to the same influence for reneurotisation, might have been prevented from reaping any important benefit from the fertile state of the cicatrix, merely on account of the proximitous presence of the healthier sheaths. As a reason for this it is pointed out that immediately after the fusion the good fibres below the neuroma, although obliged to undergo complete degeneration, nevertheless continued to contain myelin and axis cylinders for some little time. Although these nerve substances became more and more broken up, they might very well have exerted some influence, which, were it ever so slight, might nevertheless have been sufficient to have offered the initial attracting impulse for directing the growth of sprouting nerve ends after they left the scar. This view is consistent with that of Forssmann (9), who offered evidence to show that brain substance interposed experimentally in the paths of regenerating nerves seemed to offer a peculiar attraction for them. Although Forssmann admitted that mechanical influences were important in helping to control the direction of growth, he believed that this factor of specificity of nervous substance was of no lesser import.

Whether or not the chemotactic influence is an important one in determining the selection of fibres for reneurotisation, there is another basic factor which is to be considered, and that is the actual state of the peripheral end-organs. It is commonly supposed (10) that structure goes hand in hand with function, and this law might be especially applicable in cases of nerve degeneration and regeneration. Such fibres as have recently supplied actively functionating end-organs are probably in a more favourable state for reneurotisation than those that have been out of commission some time, and if these end-organs ever fall off beyond a certain point owing to lack of use, this fact would in itself preclude the possibility of a restoration of their nervous connection with the spinal centres.

## CONCLUSIONS.

(1) Nerves containing degenerated tracts do not have their anatomical pattern much altered by the interposition of surgical neuromata produced by nerve fusion; the empty sheaths do not become refilled.

(2) In the neuromata themselves marked distortion of pattern may occur, as in cases where normal nerves have been fused, but this is only a local thing and does not affect the anatomical pattern of the nerves below it. At the same time, such fibres as were not affected by the original lesion may form new central connections.

(3) Taken all in all, these experiments give us data which are reconcilable with recognised views on nerve regeneration and chemotaxis. This being so, the evidence is to be regarded as rather unfavourable for justifying nerve anastomosis after lesions in the cord or roots, unless possibly the time-interval between the original lesion and the anastomosis is very short.

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# THE OCCURRENCE OF NATIVE ANTISHEEP AMBOCEPTOR IN HUMAN SERUM AND ITS IMPORTANCE IN THE PERFORMANCE OF THE WASSERMANN REACTION*

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That there occurs in certain human serums a substance which hemolyzes sheep corpuscles in the presence of complement has been known for a considerable time. It has been our purpose to find out how much the presence of this substance in serums which reacted positively to the Wassermann test could affect the final result of the reaction.

In 1908 Bauer[1] pointed out that use might be made of this substance — to which we shall refer as native antisheep amboceptor — in the complement fixation test for syphilis, and he suggested substituting this native amboceptor for the rabbit sheep amboceptor used in the Wassermann reaction. The Wassermann technic would be simplified in this way, as one of the reagents would be eliminated without endangering its accuracy. This procedure is known as the Bauer modification of the Wassermann reaction. Shortly after this, Bering[2] reported a series of 123 cases, in which both the Wassermann reaction and the Bauer modification were used. He found that the results of the two reactions checked in all of the 123 cases, but other observers who compared the two methods found that the presence of this native antisheep amboceptor was too inconstant to be depended on. Hoehne[3] found that at least 10 per cent. of the adults had in their serum either none of this substance or an insufficient amount for the reaction. Meirowsky[4] found Bauer's modification to be inaccurate and unreliable as compared with the Wassermann reaction. Swift[5] compared the results of the Bauer reaction with the Wassermann and Noguchi reactions in sixty-five serums. He found sufficient native amboceptor to carry out the Bauer modification in 38 per cent. of the cases, and an insufficient amount in 60 per cent. of the cases. Noguchi[6]

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*From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University, Cleveland. Received for publication Feb. 5.

[1] Bauer, J.: Deutsch. med. Wchnschr., 1908, xxxiv, 698; Berl. klin. Wchnschr., 1908, xlv, 834.

[2] Bering, F.: München. med. Wchnschr., 1908, xlviii, 2476.

[3] Hoehne, F.: Berlin. klin. Wchnschr., 1910, xlvii, 334.

[4] Meirowsky, E.: Berl. klin. Wchnschr., 1909, xlvi, 152.

[5] Swift, H. F.: The Archives Int. Med., 1909, iv, 376.

[6] Noguchi, H.: Jour. Exper. Med., 1909, ix, 392.

found that this native antisheep amboceptor varied much in different serums; in some instances the amboceptor content was as higs as 20 units. Kaliski[7] found from slight to large amounts in about 53 per cent. of human adults. The above observations go to show that while there is in certain human serums enough antisheep amboceptor to hemolyze the amount of sheep cells used in the Wassermann reaction, this is by no means a constant characteristic.

Morgenroth and Sachs,[8] Noguchi[9] and others have shown that with a constant amount of red cells, hemolysis will occur when the amounts of amboceptor and complement are varied inversely to each other. In other words, hemolysis will occur with a constant amount of sheep corpuscles even if less than one unit of complement be used when the amount of amboceptor is increased proportionately. It is therefore conceivable that in a serum which contains enough native amboceptor for the purposes of a complement fixation test, the addition of the usual dose of artificial amboceptor might well change a positive reaction into a negative one by causing hemolysis to take place where none should occur.

In order to obtain an idea of the amount of native antisheep amboceptor in human serums which were to be tested by the Wassermann reaction, Swift[5] suggested the addition of a new control tube to the Wassermann system. This tube would contain one-half the amount of human serum used in the test, with the usual amount of complement and sheep-cell emulsion. He thought that if hemolysis occurred in this control tube "it would give a clue to the cause of negative findings with certain serums and would justify repeating the reaction with the Bauer modification."

Acting on this suggestion we have controlled a large number of Wassermann reactions with this extra test. We have used the modified Wassermann system recommended by Swift,[5] in which one-half the amount of serum and reagents employed in Wassermann's original technic are used. The tube containing only the patient's serum, complement and cell emulsion, which we may call the Bauer control tube, is incubated for one hour, during which time the reactions undergo their first incubation. If at the end of the hour the Bauer control shows complete hemolysis, it has been our custom to perform the test without the addition of the artificial amboceptor, depending on the native amboceptor to carry out the reaction.

We have controlled 1,686 reactions in the above manner and have found sufficient native amboceptor in 301 serums (17.9 per cent.), while in 1,385 (82.1 per cent.) there was either none or an insufficient amount.

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7. Kaliski, D. J.: The Archives Int. Med., 1910, vi, 205.
8. Morgenroth, J., and Sachs, H.: Berl. klin. Wchnschr., 1902, xxxix, 817.
9. Noguchi, H.: The Serum Diagnosis of Syphilis, J. B. Lippincott Co., 1910.

We have also reviewed our notes on ninety-five additional cases in which two or more Wassermann reactions had been done. In sixty-two of these cases there was no native antisheep amboceptor at any time. Five showed a sufficient amount of native amboceptor constantly on two or more observations, while twenty-eight cases showed an insufficient amount of native amboceptor on one occasion and a sufficient amount on others, or vice versa. These observations were made over periods of time varying from weeks to months, and merely suggest that the native antisheep amboceptor in human serum is a distinctly inconstant body even in one and the same individual.

Table of Positive Reactions Changed by the Addition of Artificial Amboceptor*

| No. | Without Artificial Amboceptor | With Artificial Amboceptor | No. | Without Aftificial Amboceptor | With Artificial Amboceptor |
|---|---|---|---|---|---|
| 1 | +++ | ++ | 15 | +++ | — |
| 2 | +++ | ++ | 16 | +++ | — |
| 3 | +++ | ++ | 17 | ++ | + |
| 4 | +++ | ++ | 18 | ++ | + |
| 5 | +++ | ++ | 19 | ++ | ± |
| 6 | +++ | ++ | 20 | ++ | ± |
| 7 | +++ | ++ | 21 | ++ | — |
| 8 | +++ | + | 22 | ++ | — |
| 9 | +++ | + | 23 | + | ± |
| 10 | +++ | + | 24 | + | ± |
| 11 | +++ | ± | 25 | + | — |
| 12 | +++ | ± | 26 | + | — |
| 13 | +++ | ± | 27 | + | — |
| 14 | +++ | ± | 28 | + | — |

*In this table +++ means strongly positive (no hemolysis); ++, positive (distinct hemolysis without visible diminution in the layer of cells at bottom of tube); +, feebly positive (hemolysis with some diminution in the layer of cells at bottom of tube); ±, plus-minus (marked hemolysis with only a faint layer of cells at bottom of tube); —, negative (complete hemolysis).

As already stated, the main purpose of the observations discussed in this paper was to find out how far positive Wassermann reactions in serums which showed a sufficient native antisheep amboceptor content could be affected by the addition of the usual dose of artificial amboceptor. In the series specially devoted to this purpose, our procedure has been to perform two separate reactions on such serums. One reaction was carried through without the addition of artificial amboceptor, i. e., with the Bauer modification, while the second reaction received the usual dose of artificial amboceptor. We have done a large number of reactions in this manner, of which seventy-seven serums showed a positive reaction to the Bauer modification.

Of these seventy-seven positive reactions forty-nine, or 63.6 per cent., remained unchanged by the addition of artificial amboceptor; while twenty-eight, or 36.4 per cent., were changed by the addition of artificial amboceptor.

Of the forty-nine reactions which were unchanged, forty-one were strongly positive (+ + +); four were positive (+ +) and four were feebly positive (+).

Of the twenty-eight reactions altered by the addition of artificial amboceptor, sixteen were classed as strongly positive. Of these sixteen reactions seven were changed to positive (+ +); three were changed to feebly positive (+); four were changed to plus-minus reactions (±) and two became negative (—) (see table).

There were six reactions classed as positive. Of these two were changed to feebly positive reactions (+); two were changed to plus-minus reactions (±) and two became negative.

There were six reactions classed as feebly positive. Of these two became plus-minus reactions, and four became negative.

Further, there were eight reactions not counted in the above series, classed as plus-minus reactions, all of which became negative.

## CONCLUSIONS

Of the twenty-eight positive reactions which were altered by the addition of artificial amboceptor, eight were changed to frankly negative reactions and eight became plus-minus reactions, which could not be considered positive. Thus it is interesting to note that out of the seventy-seven serums observed, there were sixteen positive reactions (21.8 per cent.) which became either entirely negative or ambiguous and for practical purposes could only be considered negative.

It seems justifiable to conclude that while the presence of a native antisheep amboceptor in human serum is by no means constant, it is essential to take note of its presence or absence in performing the complement fixation test for syphilis by the Wassermann method, and that if this precaution be not taken, a certain and by no means very small percentage of positive reactions will be recorded as negative. By following this simple method of controlling the reaction, this error will be obviated to a large degree.

The Rose Building.

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# THE METAMORPHOSIS OF THE ENDOSTYLE (THYROID GLAND) OF AMMOCOETES BRANCHIALIS (LARVAL LAND-LOCKED PETROMYZON MARINUS (JORDAN) OR PETROMYZON DORSATUS (WILDER)).*[1]

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PLATES 66 TO 70.

## INTRODUCTION.

Wilhelm Müller in 1873 homologized the endostyle, or hypobranchial groove, of the Tunicate, Amphioxus, and the larval Petromyzon with the thyroid gland of all higher chordates. Subsequent investigations have served to strengthen this remarkable homology, which would have been impossible of demonstration but for the survival of a single class of vertebrates, the Cyclostomes, of which the Petromyzontidæ, or Lampreys, are the best known order. The lamprey embraces in its own life history both the fullest development of the endostyle mechanism[2] and the characteristic ductless

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[1] I am indebted to Professor B. F. Kingsbury for the privileges of his laboratory and for helpful criticisms and suggestions. I wish also to thank Professor S. H. Gage for helpful suggestions, and Mr. J. F. Badertscher for aid in many technical problems.

[2] In addition to the ventral midline subpharyngeal glandular structure, or endostyle proper, there are accessory structures directly continuous with the endostyle epithelium developed in the pharyngeal mucosa that are believed to function as the path of conduction for the slime cord issuing from the orifice of the endostyle. This function has not, however, been observed in the Ammocoetes and is based on reasoning by analogy from Giard's (Giard, A., *Arch. de zoöl. expér. et gén.*, 1872, i, 525; 1873, ii, 481) and Fol's (Fol, H., *Morphol. Jahrb.*, 1874, i, 222) experiments with the endostyle structure of Tunicates, where they observed the cord of mucus issuing from the endostyle to pass forward, arch up around the sides of the pharynx to the dorsal midline, then pass back along this line to the esophagus. In the Ammocoetes the anterior part of this structure begins at the anterior margin of the large elliptical orifice of the

thyroid common to all Gnathostomes. The endostyle persists throughout the larval period of the animal's existence in full activity,[3] then at the metamorphosis, which was first made known to the zoölogical world by August Müller,[4, 5] the endostyle undergoes involution, all but a remnant disappearing, and other profound organic changes occur. This remnant assumes the form of ductless thyroid follicles and persists throughout the adult life of the lake lamprey (*Petromyzon dorsatus*) as a true ductless thyroid. It has been stated by some observers that the thyroid follicles may disappear entirely in old sea lampreys (*Petromyzon marinus*).

It is the purpose of this paper to record in some detail the series of changes that take place in the endostyle at metamorphosis and to

endostyle with which it is continuous and just posterior to the third gill arch pillar (figures 1a, 1b, and 1c). At the beginning it is a deep slit-like fold of the pharyngeal floor and roughly of the shape of an inverted T. Just anterior to the third pillar the groove widens and about the middle of the second gill pouch divides into right and left arms which continue forward with slight divergence to a point just anterior to the second gill arch pillar where the divergence becomes more marked. These grooves become shallower as they pass forward, outward, and upward around the branchial sac at its most anterior part to join the prominent dorsal midline fold where they fuse with the ciliated epithelium on the sides of this pendant-like fold and continue back along the midline to the esophagus. The ventral surface of this dorsal ridge or fold is covered with stratified squamous epithelium out of which the permanent esophagus will be formed. The posterior portion of the structure begins at the posterior lip of the endostyle orifice in the midline and extends backward along the midline of the pharyngeal floor as a shallow and narrow groove lined with ciliated epithelium continuous with that of the endostyle. This groove gradually gets smaller and narrower to end at the entrance of the larval esophagus.

The structure, then, that makes up this accessory endostylar mechanism is (1) the large, ventral midline duct-like ciliated groove issuing from the anterior lip of the gland orifice which divides into (2) two peripharyngeal ciliated grooves which terminate in the dorsal midline in the (3) ciliated dorsal ridge and (4) the shallow, narrow ventral midline groove from the posterior lip of the gland orifice to the esophagus.

[3] This persistence lasts at least for three, and possibly for four years, according to Gage (Gage, S. H., The Lake and Brook Lampreys of New York, Wilder Quarter-Century Book, Ithaca, 1893, 421).

[4] Müller, A., *Arch. f. Anat. u. Physiol.*, 1856, 323; *Ann. and Mag. Natural Hist.*, 1856, ser. ii, xviii, 298.

[5] According to von Siebold (von Siebold, K. T. E., Die Süsswasserfische von Mitteleuropa, Leipzig, 1863) this metamorphosis was known to Leonhard Baldner, a fisherman of Strassburg, in 1666.

ascertain what type or types of the endostylar epithelium persist as the lining epithelium of the ductless thyroid follicles, with the hope that it may serve as a step toward some clue to the thyroid function in this, which is seemingly the simplest form of the ductless thyroid.

## THE NORMAL ANATOMY OF THE ENDOSTYLE.

The endostyle in the course of its development in the Ammocoetes acquires a complicated morphology which in section is not easily made out. On this account I have reconstructed the organ from a brook lamprey (*Lampetra wilderi*) in the collection of Professor S. H. Gage, of Cornell University[6] (Figures 1a, 1b, 1c, and 2).

This very prominent gland in the Ammocoetes lies just beneath the pharyngeal mucosa in the ventral midline and is contained within the cartilaginous framework of the branchial sac. It extends from the beginning of the first branchial pouch to the posterior border of the fifth branchial pouch and opens into the branchial sac by a large slit-like orifice just posterior to the third gill arch pillar. This orifice is situated in the ventral midline approximately at the middle of the long axis of the gland and is continuous with the posterior end of the deep pharyngeal groove. The gland is composed of right and left symmetrically opposed halves (figure 4). Each half consists of a single straight chamber anterior to the pharyngeal orifice (figure 3a). At the anterior border of the orifice each of the anterior chambers divides into an external and internal chamber (figure 3b). Each posterior arm contains approximately one half of the epithelial investment of the corresponding anterior chamber. The two external chambers extend directly posterior, and behind the posterior border of the coil of the internal chambers curve into the midline to extend a short way back side by side and end blindly just posterior to the bifurcation of the ventral aorta (figure 3c). The internal chamber of each half of the gland extends posteriorly side by side to a point approximately two thirds of the distance between the orifice and the posterior extremity of the two lateral chambers, then curves upward and forward, and reaches the vicinity of the gland orifice where it curves downward and backward and again upward

[6] The total length of specimen 15 T, fourteen months old, was 39 mm. The total length of the endostyle was 3.08 mm.

and slightly forward to end blindly (figures 1a, 1b, 1c, and 2). Thus it describes about one and one half elongated coils. These two internal or coiled chambers if extended would be longer than the two lateral chambers. I have spoken of these coiled internal chambers as if they were separate, but they are not strictly separate chambers since the septum is not complete. They are separate, however, in that they arise from opposite halves of the organ. The median septum is complete anterior to the orifice, while at the orifice it becomes partial and remains so throughout the extent of the internal or coiled chambers. The anterior chambers then communicate with each other only at the orifice. The two lateral chambers posterior to the orifice also communicate only at the orifice, and the two internal chambers communicate throughout their length on account of the incomplete median septum. All chambers communicate with each other at the orifice.

The gland is contained within a well formed fibrous capsule which anterior to the orifice is composed of an outer layer surrounding both halves and an inner layer which passes into the septum. Posterior to the orifice the outer fibrous investment of the whole organ is well developed, but the septa dividing the four chambers are very atrophic.

The ventral aorta is continued forward in the midline from the conus arteriosus as a single tube on the dorsal surface of the posterior extension of the lateral chambers until it reaches the posterior edge of the coiled inner chambers. Here it divides into two trunks which are continued forward on either side of the coiled chambers and on the dorsal surface of the lateral chambers to the anterior end of the endostyle where they terminate in the first pair of gill arteries.

*The Lining Epithelia.*—Anterior to the orifice the most striking feature of the interior of each chamber is the large visceral invagination, or infolding (flask-shaped in transverse section (figure 3a)), from the ventrolateral wall carrying blood vessels and stroma, but composed for the most part of four large bundles (fan-shaped in cross section) of large cuneiform cells, two on each side of the midline of the invagination. These fan-shaped columns extend the entire length of the organ and are composed of long wedge-like cells with large nuclei lying at the base of the cells; *i. e.*, at the periphery of the columns. These long cells taper to a central core which opens on to the free surface by a small pyramidal projection. The finer cytology of these cells is not easily made out owing to the difficulty of obtaining good fixation. The nuclei are large, round, and vesicular. The cytoplasm also stains with basic dyes and one gets the impression that faintly staining granules and longitudinal striations are present, but, as Schneider remarks, this may be due to cytolysis. Each bundle is surrounded by a separate fibrous investment. These four bundles of gland cells make up the bulk of the interior of the invagination.

Covering the surface of the visceral invagination and thence reflected on to the septum and lateral wall of the chamber, one can distinguish four other types of epithelial cells. First on either side of the four small pyramidal openings of the large bundles there is a zone of narrow high columnar epithelium with long densely packed cilia. The nuclei of these cells stain densely, are much elongated, closely packed, and situated near the middle of the cell bodies. The cytoplasm stains with acid dyes. Extending up to the neck-like constriction of the visceral invagination is the second type of epithelium,—high columnar, with coarser and shorter cilia. These cells are of the same length as those just described, but are broader. The nuclei are round, pale, and vesicular, and lie in the basal third. The cytoplasm stains a pale red with eosin and in addition contains large quantities of a yellow flocculent and granular pigment. The knob-like apex of the fold anterior to the orifice is covered with a third type directly continuous with the second type and composed of low columnar cells with short coarse cilia. The cells have darkly staining elongated nuclei situated about the middle of the cell bodies, and the cytoplasm, while taking acid dyes, lacks the yellow pigment of the second type. This type is continuous at the orifice with that lining the pharyngeal grooves. A small zone of this same type of epithelium also occurs at the base of the visceral invagination on each side and merges into the fourth type of epithelium which covers the parietal walls, septum, and lateral wall. This area is covered with low cuboidal or flattened endothelial-like cells arranged in a single layer, as are also all the other epithelia.

At the orifice, as stated above, each anterior chamber divides so that half of the flask-shaped invagination of each anterior chamber continues in each of the four posterior chambers, the outer half of each invagination continuing as the lateral chambers, and the inner halves going into the internal, or coiled, chambers (figure 2). There is no change in the types of epithelia, each posterior chamber containing all the types found in the anterior chambers, but the number of cells is reduced approximately by half.

The glandular portion of the endostyle then contains five distinct types of epithelium: first, the four large bundles of cuneiform cells of each half of the gland; second, the narrow, columnar, densely nucleated and densely ciliated cells on each side of the pyramidal openings of the cuneiform bundles; and third, the broad columnar cells with round pale nuclei containing abundant yellow pigment granules in the cytoplasm. All these forms are confined wholly to the visceral invagination. The fourth form occurs on the knob-like apex of the visceral folds in the anterior chambers which at the orifice fuses with the parietal epithelium and at the angles of the base of the fold merges into the parietal epithelium. These cells are also columnar, coarsely ciliated, with dense elongated nuclei, and contain none of the pigment of the third type. The fifth type forms the parietal lining of the chambers, and is composed of a single layer of large endothelial-like cells.

These five types of epithelia are quite distinct and offer no difficulty of recognition by ordinary methods. I have given a detailed description of these five types because of their importance in the changes associated with the metamorphosis. In order to avoid repetition in the descriptions of the fate of each type of epithelium from the inception to the completion of metamorphosis, the five epithelia will be designated as types as follows (figure 5):

*Type I.*—The large fan-shaped bundles of cuneiform cells.

*Type II.*—The narrow densely nucleated and ciliated high columnar cells extending for a short distance on each side of the narrow openings of the cells of type I.

*Type III.*—The broad columnar ciliated epithelium with yellow granular pigment.

*Type IV.*—The low columnar ciliated cells occupying three sites: (1) the angle between the visceral fold and the parietal wall; (2*a*) the similar angle at the apex of the visceral fold posterior to the orifice; and (*2b*) the knob-like free apex of the visceral fold anterior to the orifice.

*Type V.*—The flat epithelium lining the parietal walls and septum.

## THE METAMORPHOSIS.

I have found no publications dealing specifically with the metamorphosis of the endostyle, although several observers have referred to it in a general way. Rathke[7] was the first to describe the endostyle of Ammocoetes as a gland, and in 1856 August Müller[8] observed that it disappeared at the metamorphosis. He thought that the structure became the muscular portion (tongue) of the sucking apparatus. Schneider[9] gives the first account of the metamorphosis of the endostyle as ascertained from two metamorphosing specimens of *Ammocoetes branchialis* given him by von Siebold. He noted that the large bundles of cuneiform cells disappeared during the change, while the connective tissue framework and lining ciliated epithelium remained. He stated that the fibrous tissue increases and forms a dense wall about the epithelium and gradually compresses it, although the epithelium holds to its orderly arrangement in the tube until at last the fibrous tissue growth cuts the tubules into segments and thereby obliterates all its original endostylar structure and creates the ductless thyroid follicles. W. Müller in his earlier paper,[10] while recognizing the thyroid gland in the adult *Myxine glutinosa,* seems to have mistaken the salivary gland for the thyroid in *Petromyzon fluvialis.* In a later paper[11] he states that only at metamorphosis the large glandular endostyle atrophies and only a small remnant of its epithelial lining remains as the ductless thyroid follicles of the sexually mature lamprey.

The extensive papers of Schneider's pupil, Kaensche,[12] and of Bujor,[13] give no account of the changes taking place in the endostyle at metamorphosis.

My observations are based on the study of twenty-four metamorphosing specimens taken from the mud and sand of Cayuga Lake Inlet at Ithaca, New York. Specimens for microscopic study were taken from the stream twice each week from June 17 to August 11, 1912.

Metamorphosing specimens in excess of those needed at the time of taking were stored in laboratory aquaria which were half filled with mud and through which running water was continually passing. After August 11 one of these laboratory specimens was removed every fifth day until September 20. I was able therefore to study an unbroken series from June 17 to September 20, under natural conditions until August 11, and modified by laboratory residence from August 11 to September 20.

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[7] Rathke, M. H., Bemerkungen über den inneren Bau des Bricke, Danzig, 1826.

[8] Müller, A., *loc. cit.*

[9] Schneider, A., *Ann. and Mag. Natural Hist.*, 1873, ser. iv, xi, 236.

[10] Müller, W., *Jenaische Ztschr.*, 1871, vi, 428.

[11] Müller, W., *Jenaische Ztschr.*, 1873, vii. 327.

[12] Kaensche, C. C., *Zoöl. Beitr. von A. Schneider*, 1890, ii, 219.

[13] Bujor, P., *Rev. biol. du nord de la France*, 1890–1, iii, 301; 1891–2, iv, 41.

The first specimen showing external signs of metamorphosis was taken on July 19. In a specimen taken July 16 and showing no external signs of metamorphosis, microscopic examination revealed in the endostyle changes that in the light of subsequent studies of the series as a whole must be considered as the earliest evidences of metamorphosis; namely, a thickening of the fibrous capsule and a slight reduction in the volume of the cells of type I. The specimen of July 19 shows, on careful examination, external changes. The dentaphores are visible as a stipple-like roughening on the inner surface of the upper leaflet of the hood and there is partial obliteration of the normal sharp angle at the juncture of the upper and lower leaflet of the buckle hood. The eyes could not be seen in the fresh specimen, but after a day's fixation in formalin, round whitish spots in the location of the eyes were visible through the skin. The prominent endostyle changes are the same as, but slightly more marked than those recorded for the specimen of July 16.

The specimen of July 23 shows obvious external changes. The body length is 14.7 cm., the eyes are clearly visible, the color is slate gray on the dorsal surface, and brownish yellow on the ventral surface. Dentaphores are visible, and there are slight elongation of the head and partial obliteration of the angles of the buccal hood.

The endostyle (figure 6) has undergone a striking reduction in size, measuring 1.006 mm. in diameter at the level of the orifice, as compared with 1.518 mm. for the normal endostyle of an ammocoetes of the same size. The fibrous capsule is sharply outlined as a thick band closely investing the gland and involving the septum as well. There is also a new formation of fibrous tissue in the periglandular zone. The interval between the gland and the pharyngeal mucosa is increased by this new tissue formation which is in the location of the future tongue. Of the epithelia, the cells of type I are reduced one third of their original volume, although they retain their wedge shape and arrangement. The nuclei of these cells have migrated from the base of the cells, *i. e.*, the periphery of the fan-shaped formation, and occupy the middle third of the cells. All four columns are affected to about the same extent. The cells of types II, III, and IV are somewhat compressed, but their general appearance is but little altered. The cells of type V have become more cuboidal owing to reduction in the size of the chambers.

The specimen of July 26 is 14.4 cm. in length. The eyes are distinct, the nasal pore large and patent, and the pineal eye white. The head and branchial region appear reddish and semitranslucent from the increased vascularity. The color on the dorsal surface is a dark slate gray and on the ventral surface it is tinged with yellow. The endostyle measures 0.627 mm. in diameter at the level of the orifice and exhibits reductions in the volume of all its component parts (figure 7). The sharp line of demarcation between the thickened capsule and the newly formed tissue external to it, which was a prominent feature in the specimen of July 23, is absent and the two tissues merge. The whole area is much more vascular and contains large blood spaces. The tongue anlage is visible as a rounded bundle of large closely set elongated cells. The cells of type I are still detectable in the fan-shaped areas, although connective tissue has begun to invade them. The cell boundaries are for the most part lost. There is clumping of the cell masses. The nuclei are scattered irregularly through the area and present many stages of dissolution. The surface connection of these cell bundles is lost although the site of this connection is recognizable as slight notches in the cells of type II between which these bundles normally opened. The cells of type II are more compressed though still ciliated and high columnar. The nuclei of these cells while normally dense and closely set are through compression so closely packed that their limiting membranes are barely visible. The cells of type III have undergone a great reduction in size and probably also in number. The cilia are still present. The characteristic yellow pigment granules are more in evidence, probably as a result of a more rapid destruction of the cells than of the pigment. The cells of type IV are the least affected of all the types. There is possibly some slight reduction of their volume and a loss of cilia. The cells of type V, as a result of the further shrinkage of the capsule, are more cuboidal. Their positions are slightly altered and the first evidence of piling up and overlapping is seen.

The specimen of August 1 is 12.5 cm. in length. The eyes are large and clear and produce slight disc-like prominences on the sides of the head as in the adult. The color on the dorsal surface is a dark slate gray, and that on the ventral surface is tinged with yellow. The hood is much elongated. The sucking disc is now complete and all outlines of the larval hood are absent. The pharyngeal grooves can still be made out and the gland duct, much elongated, is still patent notwithstanding the extreme atrophic changes in the endostyle. The pericapsular tissues are vascular. The dense fibrous tissue of the thickened capsule, so evident in specimens of July 16, 19, and 23, has disappeared and the rather loose, palely staining fibrous tissue extends to the gland epithelium lining the chambers. The septum between the two halves of the endostyle is now a wide band of proliferating fibrous tissue separating the two halves as well as rotating their dorsal portions outward. At the level of the orifice the gland diameter is 0.462 mm. The individual chambers anterior to the orifice are about 0.2 mm. in diameter, or slightly smaller than the diameter of each ventral aorta at the same level.

The fan-shaped bundles of type I are no longer distinguishable as such. Connective tissue ingrowths from the base of the visceral fold and from the fibrous tissue within the fold itself have broken up these areas so that one sees only shrunken and distorted cell masses with or without visible nuclei (figure 8). These areas are, in general, lighter in color. The cells of type II are still ciliated, and are considerably reduced in length and extent. All evidence of the openings of the cells of type I is gone. The nuclei are basal, very dark, and closely packed. The cells of type III are much reduced in size and number. The yellow pigment granules are relatively more abundant and are contained for the most part in the basal portion of the cell bodies, but are also present above the nucleus. The scattered cilia are still to be made out on these cells. The cells of type IV are well preserved although the cilia are absent. Their extent is slightly reduced by compression. Their nuclei are large, darkly staining, and closely packed. The cells of type V, owing to the great contraction of the chambers, are irregularly distributed, piled up, in places two or more in thickness, and their nuclei show many stages of degeneration.

The specimen of August 5 is 14.9 cm. in length. The anterior portion of the endostyle is separated from the pharynx by the now large muscular anlage of the tongue which lies between the aortæ and the endostyle. The duct is elongated, and while its outline is visible, its pharyngeal connection has been closed at the level of the tongue anlage. All the pharyngeal grooves are gone and, in cross section, the branchial sac is a smooth walled oval chamber with the permanent esophagus anlage embedded above it in the dorsal midline. The endostyle chambers are still discernible in both the anterior portions as clefts, about 20 $\mu$ in width. A capsule as such is no longer visible. The fibrous tissue has become uniform so that no line of demarcation exists between the visceral fold and the parietal wall. The diameter of the endostyle at the level of the duct is 0.146 mm. All the epithelium of type I has been absorbed. The cells of type II have either atrophied beyond recognition or fused with the lower area of type IV. It appears as if this epithelium had gone, but I lack sufficient gradations to exclude a fusion. The cells of type III are now represented by a few atrophic cells containing a great abundance of yellow pigment granules (figures 9 and 10). The cells of type IV remain prominent. They are more cuboidal and their nuclei are probably paler than in previous stages. The cells of type V appear as an extruded mass of large, distorted, and disintegrating cell masses lying to the outer side of the cleft-like remnant of the chambers.

The specimen of August 9 is 12.5 cm. in length. All evidences of the true endostyle are absent in this specimen. The duct is obliterated. The small follicles and nests of epithelial cells lie in a rather uniform area of connective tissue. These follicles and cell nests are grouped into right and left bundles corresponding to the right and left halves of the old endostyle, and by the obliteration of the septum and ingrowth of fibrous tissue they are relatively widely separated. Anterior to the old orifice there are three to four follicles or nests of cells in each group corresponding to each half of the endostyle; while posterior to the old orifice and corresponding to the coiled portion of the inner chambers the follicular and cell nest remnants of the two halves of the old endostyle are closely approximated, and one can make out as many as twelve to fifteen follicles or cell nest areas. Of the epithelia, type I is completely absent, and type II is either completely absorbed or, becoming atrophic, has fused with the lower area of cells of type IV. Type III is still recognizable as a few nuclei surrounded by clumps of yellow pigment, in some levels extruded toward the lumina of the future follicles and in others pushed backward in the connective tissue stroma. Type IV is now the only prominent epithelium. The upper or dorsal area has in places assumed definite follicular shapes, utilizing the remnant of the original chamber as their lumina. In places the epithelial layer is slightly curved while in others it has completed the formation of the follicle. The lower mass of epithelium of type IV and possibly the fused cells from type II appear as a more or less curved layer of well preserved cuboidal cells surrounding the remnant of the original chambers. The cells of type V may still be made out as a small mass of more brightly staining cell remnants in the connective tissue opposite the concavity of the cell layers of type IV (figures 11, 12, and 13).

From this stage on, the further involution is largely a matter of the absorption of the remnants of the disappearing types of epithelia, the completion of the follicle formations out of the persisting type of epithelium (type IV), the development of the tongue and of the branchial sac or bronchus, the formation of the great blood spaces about the tongue through the absorption of the connective tissue, and the rotations and possible migrations, distortions, and secondary constrictions of the original follicular tubes by changes in the interfollicular stroma.

## EXPERIMENTAL OBSERVATIONS.

*Experiment 1.*—September 4, 1911. Sixty-five specimens were collected and provisionally classified as follows:

9 of the hatch of 1911, measuring 1.9 to 2 cm. in length.
34 of the hatch of 1910, measuring 6 to 6.5 cm. in length.
20 of the hatch of 1909, measuring 11 to 12 cm. in length.
2 of the hatch of 1908, measuring 14 to 15 cm. in length (metamorphosis complete).

The specimens were taken to Cleveland and placed in jars half filled with mud and sand in which some hay was placed, and running lake water was supplied continuously to each jar. On September 10 the twenty specimens of the hatch of 1909 were divided into four lots of five each and placed in separate jars. To two of the lots one drop of Lugol's solution was added daily, while the remaining two lots were kept as controls. On the 14th of each month, beginning September 14, 1911, and ending June 14, 1912, a specimen from the lots exposed to iodin and one of the controls were taken for histological examination. The object of this experiment was to see whether iodin might influence the endostyle structure and possibly afford a chemical connecting link with the ductless thyroid gland which is so markedly influenced by iodin. The results were entirely negative as regards any morphological change. This negative evidence, however, in so far as it is of value, supports the hypothesis of Müller, Dohrn, and others, based on morphological changes, that the physiology of the endostyle is quite different from that of the ductless thyroid.

*Experiment 2.*—Attempts were made to destroy the endostyle organ in ammocoetes varying in length from 10 to 16 cm., (1) by means of injecting paraffin into the gland lumen, and (2) by cauterizing with a fine electric needle. Ether vapor was found to produce a safe and quick anesthesia from which the animals rallied in a few minutes. Chloroform vapor and several solutions of chloretone and magnesium sulphate were tried, but proved to be too toxic.

The operation was performed by placing the anesthetized ammocoetes with its ventral surface up in a grooved pad of water-soaked absorbent cotton. For the paraffin injections, a fine hypodermic needle with screw adjustment for graduating the pressure was used. A mixture of paraffin oil and 52° paraffin was tried. The method was abandoned on account of the hardening of the paraffin in the needle as soon as it came in contact with the cold surface of the ammocoetes.

With the fine electric needle and the animal in the dorsal position the following procedure was carried out. The skin and musculature were burned through in the ventral midline between the second and fourth gill openings and the tip of the cautery was allowed to extend into the branchial sac. The entrance of the cautery into the sac is easily detected. The cautery was then extended slightly forward and backward from this point. This produced some destruction of the endostyle between the right and left branches of the ventral aorta in the region of the gland orifice. Ten ammocoetes were thus operated upon on July 13. All recovered and were kept until August 11, when the experiment was interrupted. The ammocoetes were all vigorous and active at the time they were killed. The cautery wounds had healed in all and the sites were marked by white, nonpigmented scars.

Histological examination of these ten specimens shows that the endostyle was only partially destroyed in each. The large bundles of cuneiform cells (type I) were destroyed at the cauterized site and exhibited no evidence of regeneration. There was an inflammatory reaction in the visceral invagination extending for a considerable distance beyond the cauterized area and containing eosinophiles. In none of the specimens were the chambers destroyed, as I hoped they might be, and it appears that an actual regeneration of at least two types of epithelium takes place: type IV, or that lining the duct and continuous with the pharyngeal grooves, and also type V, or the endothelial-like lining of the parietal walls. There is no evidence that the yellow pigment-bearing cells of type III regenerate. The duct was not destroyed in any of the specimens, although in two specimens evidence of severe injury was present, yet the epithelial lining had regenerated.

These observations, although meager, show that gross injury may be induced experimentally without death of the animal, and that some of the endostylar epithelia have little or no regenerative capacity, while others exhibit considerable regeneration within the period of a month. Further experiments extending over a longer time so as to include the effects of metamorphosis on animals which have been operated on in this way seem highly desirable.

## SUMMARY.

The first specimen of *Ammocoetes branchialis* that showed histologically any atrophic changes in the endostyle was taken on July 16. These changes proceeded relatively rapidly for about a month, after which the endostyle as such was no longer recognized. All specimens examined after August 15 showed in cross section the characteristic ductless follicles more or less completely formed. More gradual and minor changes in the way of further absorption of cell remnants and completion of the follicles continued at least until September 1. Two specimens taken from the creek on September 4, 1911, showed complete follicle formation with some stainable colloid (figures 14 and 15). There was still yellow granular pigment in the fibrous tissue between the follicles. In two specimens[14] taken on October 14, 1909, the pigment was absent and the follicles were more closely set, larger, and contained homogenous colloid. In the twenty-four specimens of ammocoetes studied, there were variations in the time of the onset of metamorphosis. There may also be variations in the rate of progress of the changes in different specimens. There is no evidence that removal of the animals from their native environment to the laboratory either increases or decreases the rate of metamorphosis. Schneider states that he was unable to get specimens kept in the laboratory to undergo metamorphosis. Gage, however, has repeatedly observed the metamorphosis under laboratory conditions, and the six of our specimens kept in the laboratory—some for forty days—remained in excellent condition and the metamorphosis proceeded as well as in those living in the creek. I know of no observations bearing on the question as to whether the metamorphosis may be hastened or delayed as it can be in tadpoles and other amphibia. It is probable, however, that physical conditions greatly influence the transformation.

These observations as to the length of time from the inception to the completion of metamorphosis indicate that a month and probably longer is necessary for the lake and brook lampreys of Central New York. This is in agreement with the observations of Gage[15] and of Müller[16] on metamorphosis in general, but is at variance with the views of Bujor,[17] who states that the process takes place within three to four days.

The first endostylar changes are a gradual shrinkage in the whole organ with thickening of the capsule and septum and proliferation of the connective tissue in the periendostylar zone. The tongue anlage is developed in this thickening just dorsal to the endostyle and anterior to the gland orifice. The size of the chambers progressively decreases and with the thickening of the septum the halves of the endostyle are both absolutely and relatively more separated.

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[14] These two specimens were presented to me by Professor Kingsbury for serial sections.

[15] Gage, S. H., *loc. cit.*

[16] Müller, A., *loc. cit.*

[17] Bujor, P., *loc. cit.*

All the five types of epithelia are affected, the first to show the change being type I, the four fan-shaped bundles of cuneiform cells of each half of the endostyle. These disappear totally quite early. The next type to show marked changes is type III, or the cells with yellow pigment granules. Here the change is progressive and these cell groups in different stages of atrophy may be traced through to the fully developed follicles. The epithelium of type V, or the endothelial-like lining of the parietal walls of the chambers, is piled up and extruded laterally as the chambers contract or shrink. These cells in different stages of atrophy may be followed until the metamorphosis is nearing completion. It is certain that the cells of types I, III, and V play no part in the formation of the ductless follicles. With types II and IV the question is not so easily settled as it is from one or the other or from both of these types that the permanent follicles arise. One can say definitely that type IV plays the major rôle, but whether the cells of type II after fusion with the basal group of type IV do not also share in the formation of the ventral follicle of the given chamber, I cannot decide, but from the evidence obtainable this seems probable. It is significant that the cells of type IV are continuous with, and indistinguishable from, the cells lining the orifice and are continued anteriorly in the deep pharyngeal groove and peripharyngeal grooves as well as posteriorly from the orifice in the small pharyngeal groove. As to the fate of this extraglandular epithelium of type IV I have no data save that with the closing of the orifice and the formation of the permanent branchial sac these grooves with their ciliated epithelium disappear and the whole sac comes to be lined with plain stratified epithelium. The fact that the cells of the pharyngeal grooves and the lining cells of the gland orifice are continuous with the cells of the endostyle from which the permanent thyroid follicles are formed is not without significance in relation to the development of the thyroid of the higher chordates. One or more very large follicles are formed from the lower portion of this orificial epithelium of type IV.

Four ductless follicles are the maximum number that may be formed primarily in each half of the endostyle from the four areas of epithelium of type IV. From the specimens studied this maximum is frequently not obtained. Posterior to the orifice where four chambers exist, each corresponding to one half of an anterior chamber, but two follicles may be formed from each chamber, but in the coil these are proportionately increased, in cross section. Most of the detailed studies here recorded have been made on the part of the endostyle posterior to the coil where the simplest conditions exist. Here two follicles are ordinarily formed from each chamber.

In cross sections the follicles are at first only long tubules whose cavities are the remnants of the original endostyle chambers, but when the metamorphosis is completed each of these primary tubules is cut up into several elongated closed sacs corresponding to the true ductless follicles of all higher chordates.[18] New follicles also arise by budding from these primary ones, and this process is probably of normal occurrence at the metamorphosis.

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[18] Dr. H. D. Reed, of the Department of Zoölogy of Cornell University, gave me twelve specimens of adult lake lampreys taken at spawning time during the spring of 1912 from the Cayuga Lake Inlet. In examining histologically the thyroid areas of these sexually mature lampreys certain features worthy of mention were noted. (1) The thyroid area still shows the division into the right and left halves corresponding to the endostyle halves. This separation is most marked in the anterior half of the thyroid area. (2) The increase in number of follicles due to the coil of the inner chambers of each half of the endostyle can be made out. (3) In two specimens the follicles were much larger and the number of follicles was increased above that which obtains just after the metamorphosis, suggesting that both hypertrophy and hyperplasia of the epithelium lining the ductless follicles have occurred. This epithelium was originally that of type IV of the endostyle. This is of interest in suggesting that these cells, after involuting to a given level as part of the destruction of the endostyle, assume a resting stage from which they may again undergo hypertrophy. (4) In one of the twelve specimens (a large adult female) the thyroid follicles were very large, some measuring 0.214 mm. in diameter, although there was no increase in the number over that obtaining just after the metamorphosis. The lining epithelium was very high columnar and for the most part, if not completely, ciliated. The nuclei of these very large high columnar cells were large, rounded, and palely stained. In each follicle there was a homogeneous red staining colloid-like mass. The significance of this observation is probably greater than one can estimate from the few facts obtainable from this accidental specimen. The fact that the endostylar epithelium from which these follicles arise is ciliated, and that these cilia usually disappear rather early in the metamorphosis, suggests that some physiological factor is involved either by way of preventing normal involution through sustained physiological activity of the larval cells, or by inducing a regeneration of the involuted cells to the larval type.

## EXPLANATION OF PLATES.

### Plate 66.

Fig. 1a. Photograph of a wax model with the pharyngeal mucosa attached, showing (*a*) the pharyngeal opening of the endostyle, (*b*) deep anterior pharyngeal groove, (*c*) branching of the anterior groove into the two peripharyngeal groves, (*d*) shallow posterior pharyngeal grooves, (*e*) left terminal branch of the aorta and (*f*) opening into branchial pouch.

Fig. 1b. Photograph of a wax model with the pharyngeal mucosa removed (dorsal view), showing (*a*) endostyle orifice, (*b*) coiled internal chambers, (*c*) left external chamber, (*d*) median raphe and septum, (*e*) conus arteriosus, (*f*) bifurcation of the aorta, and (*g*) gill arch artery.

Fig. 1c. Photograph of a wax model with the pharyngeal mucosa removed (lateral view).

Fig. 2. Photograph of transverse sections of the model, (*a*) anterior to the orifice, showing visceral invagination and the arrangement of the large bundles of cuneiform cells, (*b*) just posterior to the orifice, (*c*) through the coiled portion, and (*d*) through the posterior edge of the coil.

### Plate 67.

Fig. 3a. Photomicrograph of a transverse section of the endostyle anterior to the orifice.

Fig. 3b. Photomicrograph of a transverse section of the endostyle through the orifice.

Fig. 3c. Photomicrograph of a transverse section of the endostyle posterior to the coiled chambers.

### Plate 68.

Fig. 4a. Schematic diagram of the left half of the endostyle. The single anterior chamber branches at the anterior edge of the orifice into an inner coiled chamber and an outer straight chamber. In the diagram the inner chamber is distorted inward and rotated to the left from its normal upright position.

Fig. 4b. Composite diagram of the right and left halves with the two coiled chambers rotated outward from their normal upright positions.

Fig. 4c. Schematic diagram of the right half of the endostyle.

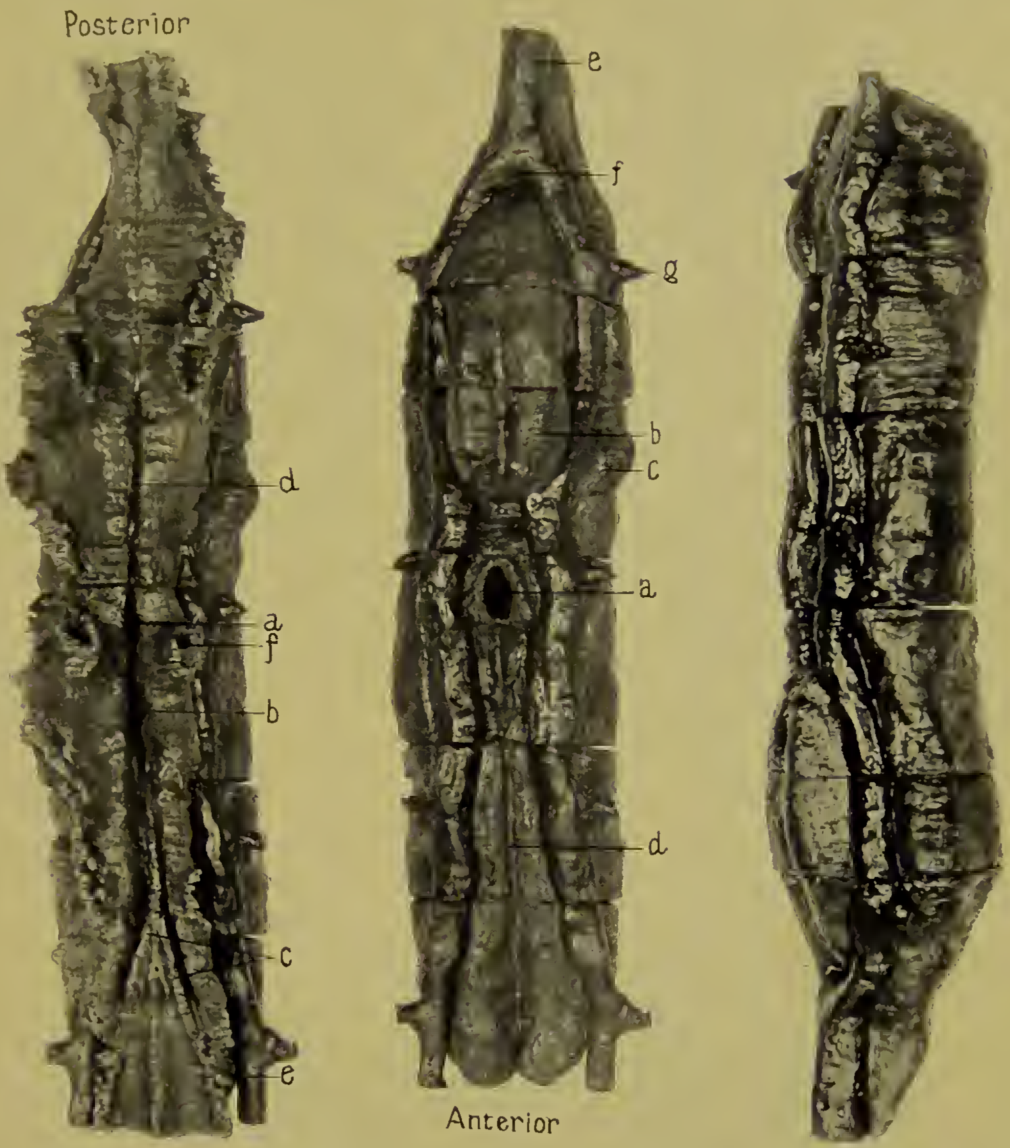
Fig. 5. Camera lucida outline of a transverse section of the left external chamber posterior to the coil, showing the arrangement and distribution of the five types of epithelium in the normal fully developed endostyle. × 107½.

Fig. 6. Camera lucida outline of a transverse section of the left external chamber posterior to the coil, showing the earliest changes in the epithelia at metamorphosis. × 367½.

### Plate 69.

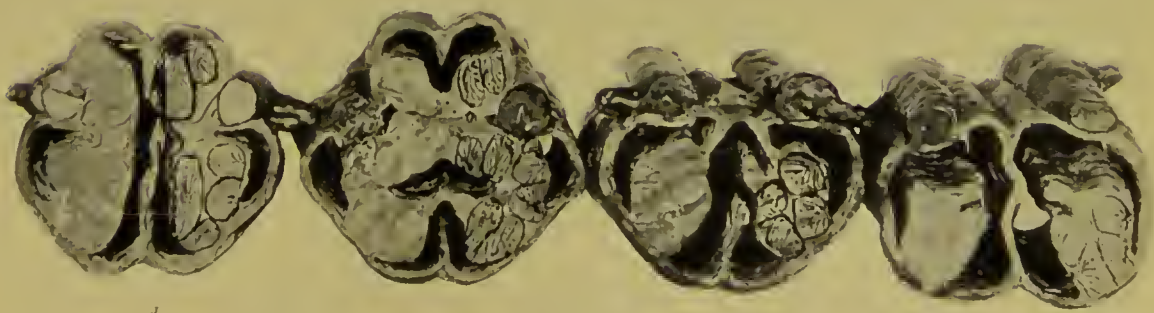
Fig. 7. Camera lucida outline of a transverse section of the left external chamber posterior to the coil, showing a slightly later stage of metamorphosis. × 367½.

Fig. 8. Camera lucida outline of a transverse section of the left external chamber posterior to the coil, showing a slightly later stage of metamorphosis. × 367½.



*a* *b* *c*

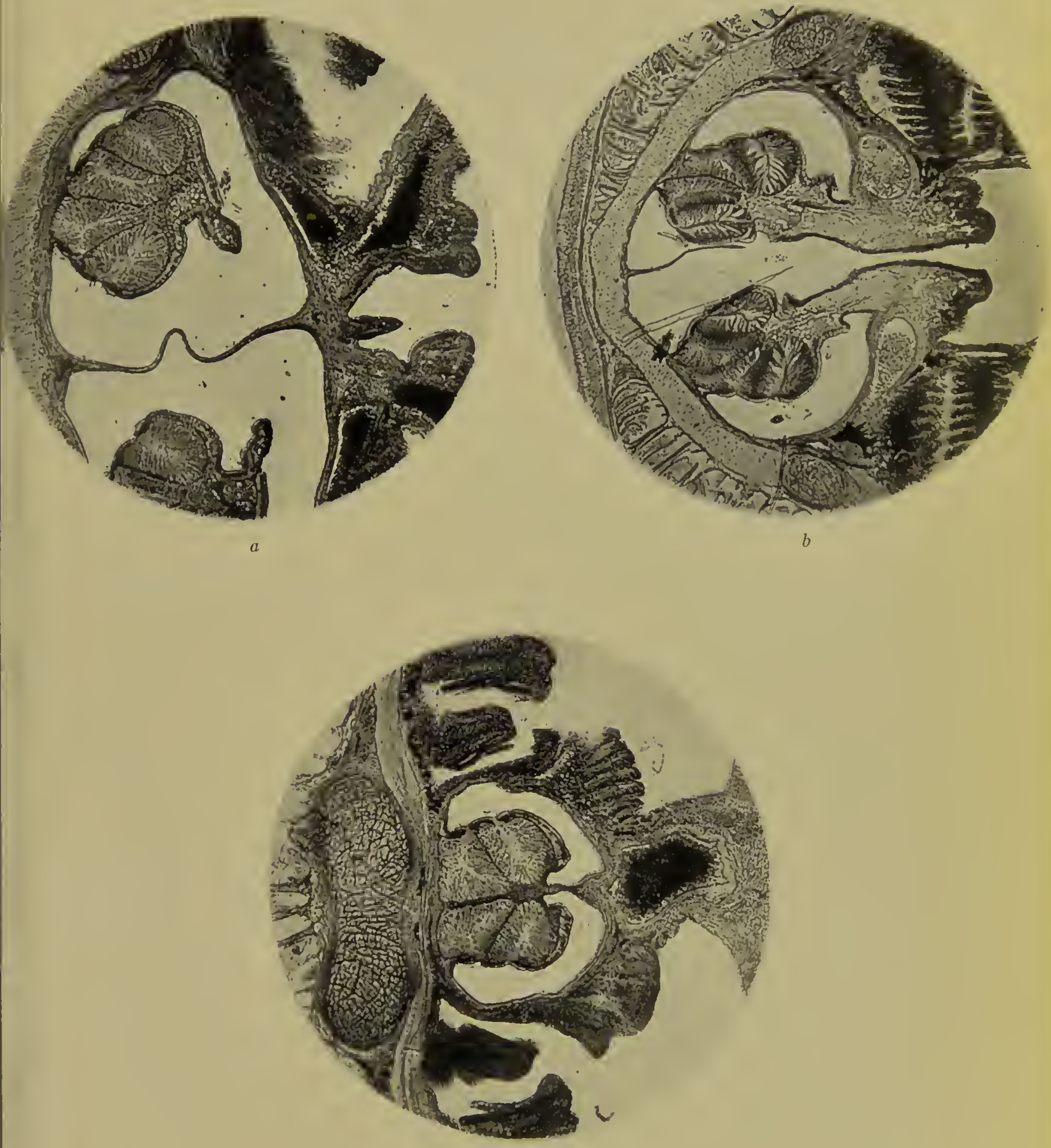
FIG. 1.



*d* *c* *b* *a*

FIG. 2.

(Marine: Metamorphosis of Endostyle of *Ammocoetes branchialis.*)



*a* *b*

*c*

FIG. 3.

(Marine: Metamorphosis of Endostyle of *Ammocoetes branchialis*.)

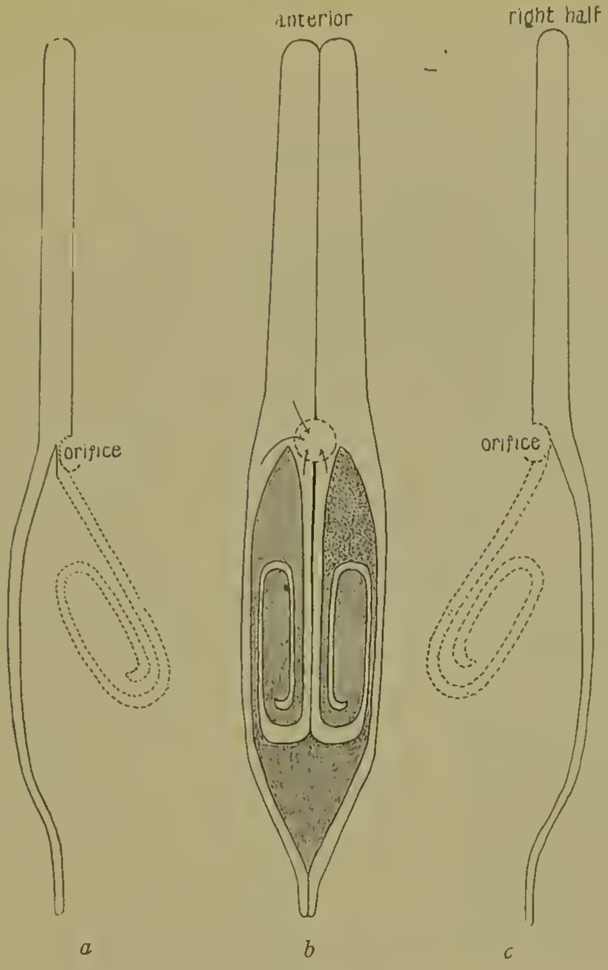


FIG. 4.

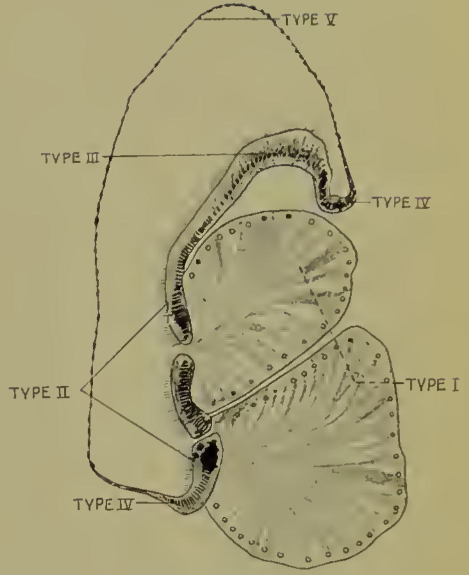


FIG. 5.

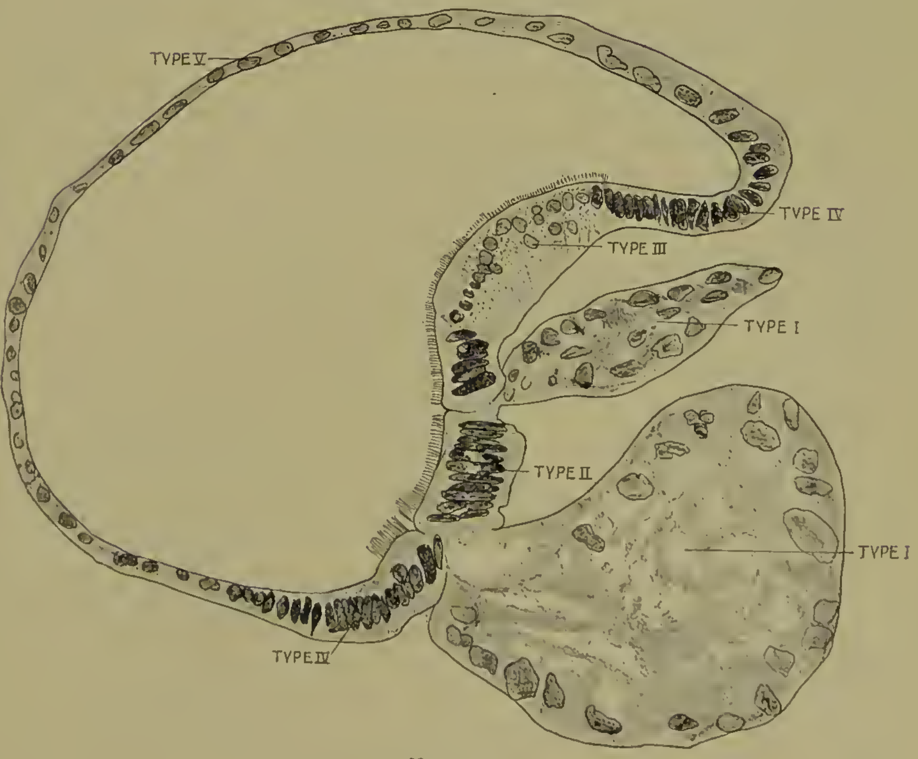


FIG. 6.

(Marine: Metamorphosis of Endostyle of *Ammocoetes branchialis*.)

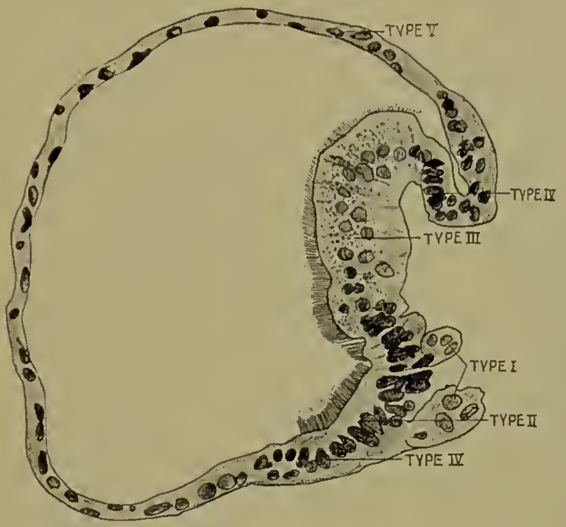


FIG. 7.

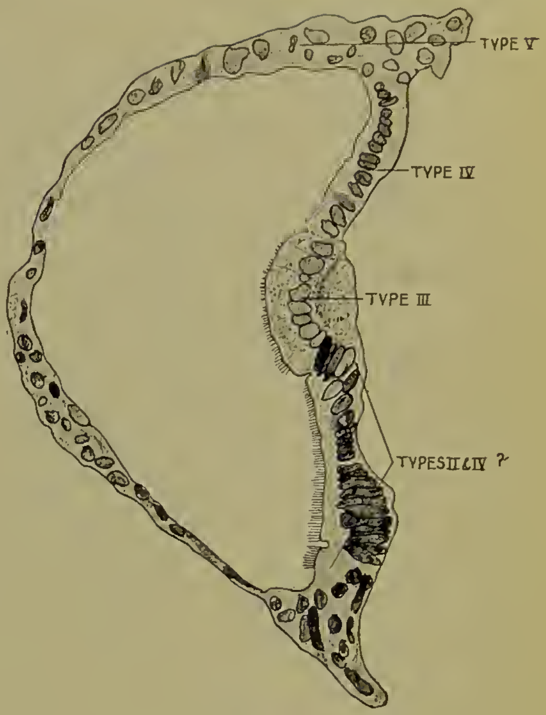


FIG. 8.

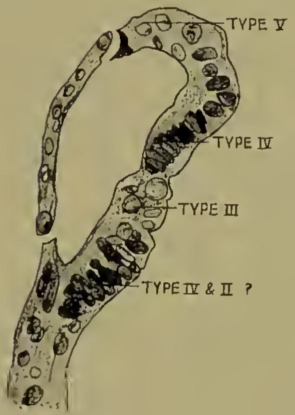


FIG. 9.

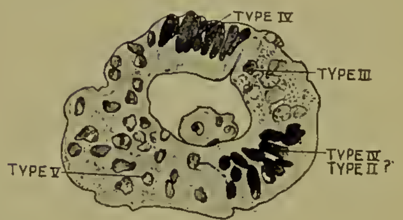


FIG. 10.

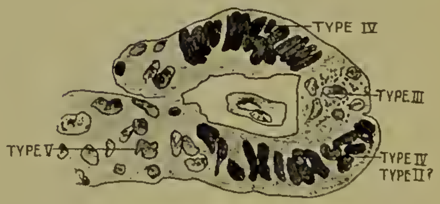


FIG. 11.

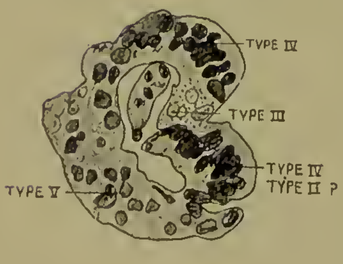


FIG. 12.

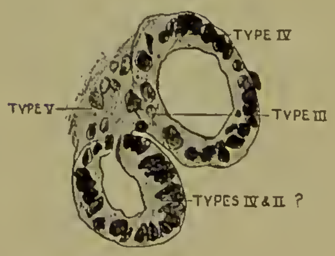


FIG. 13.

Marine: Metamorphosis of Endostyle of *Ammocoetes branchialis.*

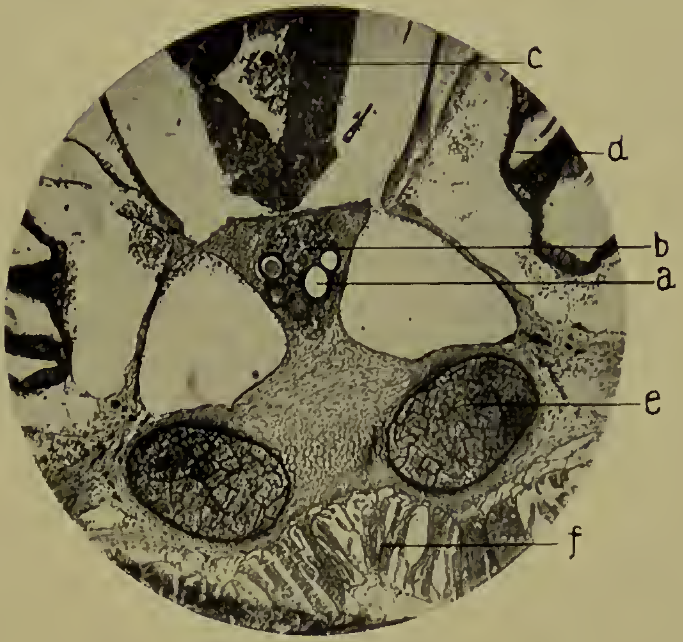


FIG. 14.

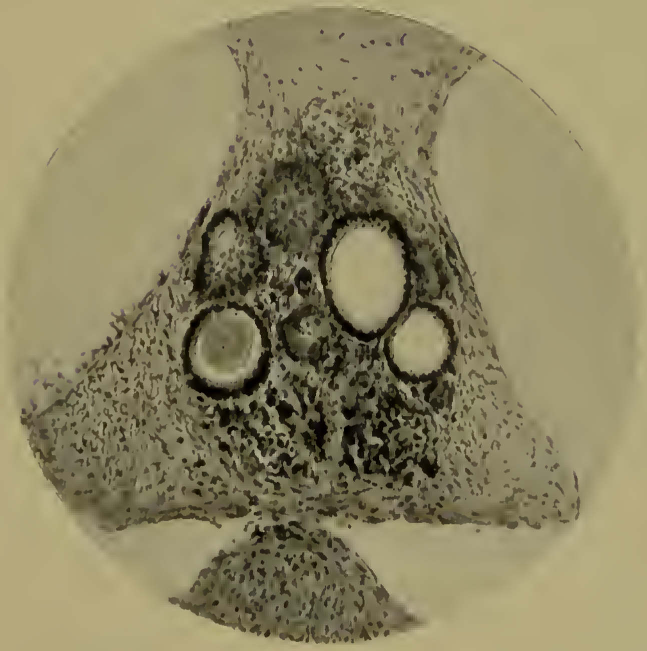


FIG. 15.

(Marine: Metamorphosis of Endostyle of *Ammocoetes branchialis*.)

Fig. 9. Camera lucida outline of a transverse section of the left external chamber posterior to the coil, showing a still later stage of metamorphosis. × 367½.

Fig. 10. Camera lucida outline of a transverse section of the left external chamber posterior to the coil, illustrating the last stages of the metamorphosis. × 367½.

Fig. 11. Camera lucida outline of a transverse section of the left external chamber posterior to the coil, illustrating a slightly later stage of the metamorphosis than is shown in figure 10. × 367½.

Fig. 12. Camera lucida outline of a transverse section of the left external chamber posterior to the coil, illustrating the earliest ductless thyroid formation. × 367½.

Fig. 13. Camera lucida outline of a transverse section of the left external chamber posterior to the coil, illustrating two distinct follicle formations from the single chamber. × 367½.

## PLATE 70.

Fig. 14. Photomicrograph of a transverse section of the thyroid area from a specimen removed from the creek on September 4, 1911, in which the metamorphosis was complete, showing (*a*) the thyroid area with complete follicle formations, (*b*) pigment and cellular debris, (*c*) tongue, (*d*) gills, (*e*) cartilaginous framework of the branchial sac, and (*f*) somatic musculature.

Fig. 15. Photomicrograph of the thyroid area shown in figure 14. Higher magnification.

# EXPERIMENTAL OBSERVATIONS ON THE EFFECTS OF THE ADMINISTRATION OF IODIN IN THREE CASES OF THYROID CARCINOMA (TWO HUMAN AND ONE CANINE)*

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There is no doubt that in the development of normal tissues structural differentiation and functional differentiation parallel each other, but in the case of tumors little is known as to whether any such parallelism exists. One obvious reason for this is that very few of the tissues from which tumors most frequently develop have any readily estimated physiological attributes. Beginnings have been made in this direction in the case of adrenal tumors (hypernephromas) through investigations for the presence of the specific pressor substance.[1] So also considerable work has been done with lipomas by way of chemical analysis and comparison with the fat of normal adipose tissues.[2] For investigations in this field it has long seemed to us that the thyroid offered most favorable conditions. Thus its architecture is simple; it is localized, easily accessible and has a wide range of morphological variations within the limits of normal functional activity. In man it is one of the most frequent sites for tumor development, particularly the adenomas. The most favorable factor, however, is that the easily determined iodin offers a fair test for the functional activity of this tissue. Thus it is well known that iodin to a marked degree controls the morphological changes associated with the physiological variations and overgrowths in the gland and also that iodin is an essential constituent of the active principle of the gland's secretion. It is on the basis of a rather extensive experience with the relation of the iodin content to gland structure and on the comparative effects of iodin on the thyroid in various phases of physiological activity that some years ago one of us undertook the study of the relation of the iodin content of benign epithelial tumors (adenomas) to their histological structure and of the effect of the administration of iodin on such tumors in comparison with its effects on the adjacent non-tumor thyroid. Some of these observations have been published.[3] Summarizing briefly it was

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*From the H. K. Cushing Laboratory of Experimental Medicine and the Department of Pathology, Western Reserve University.

*Manuscript submitted for publication Dec. 16, 1912.

[1] Brooks: Jour. Exper. Med., 1911, xiv, 550.

[2] Wells: The Archives Int. Med., 1912, x, 297.

[3] Marine: Jour. Med. Research, 1913, xxvii, 228.

found that all benign epithelial tumors have some capacity for iodin, but that this is always much below that of the adjacent non-tumor thyroid. There are variations in the capacity of these tumors for iodin which in a general way run parallel with the degree of morphologic differentiation. Thus simple adenomas or the most differentiated group have the greatest capacity, approaching closely in some instances to that of the non-tumor thyroid tissues, while the fetal adenomas, or the least differentiated have the least capacity for iodin. In the interpretation of these findings it was suggested that benign epithelial tumors of the thyroid are only partial tumors, in that they still have preserved, to some degree at least, one easily detectable, physiological attribute of the non-tumor thyroid tissue.

In view, therefore, of the well-known iodin relations in the simple non-tumor functional hyperplasias (goiter) and of the findings in the benign epithelial tumors, it seemed of importance to extend these observations to include malignant epithelial hyperplasias of the thyroid. During the past year two opportunities for such observations in man have occurred, and it is through the courtesy of Drs. C. A. Hamann and E. P. Carter, in whose services at the City Hospital the cases were admitted, that we have been able to observe and follow them to their termination. In both cases a portion of the thyroid tumor was removed several months before death, iodin was administered for a long period of time and autopsies were obtained. Abstracts of the cases follow:

### ABSTRACTS OF CASES

Case 1.—J. K., City Hospital No. 11766, admitted November 2, 1911. Male, Pole, aged 37, laborer. Owing to poor interpretation only a partial history was obtained.

Complaint: Enlargement of the neck with pain.

*Physical Examination.*—Patient was well developed and fairly well nourished. There was slight bilateral prominence of the eyes, no tremor, no tachycardia. General glandular enlargement with an increase in the size of the thyroid involving both lobes and the isthmus. The thyroid was somewhat tender on pressure and on deep palpation was slightly nodular. Admission temperature 102.3 F., pulse 80, respiration 18. Thyroiditis was suspected. Blood Examination: Hgb. 85 per cent.; R. B. C. 4,200,000; W. B. C. 8,600. Differential count: Polymorphs. 67 per cent.; mononuclears 10 per cent.; lymphocytes 17 per cent.; eosinophils 1 per cent.; transitionals 5 per cent. Urine on November 16 showed a trace of albumin.

November 5, 1911, measurement of neck in largest circumference 42 cm.

November 12, 1911, measurement of neck in largest circumference 40 cm. Temperature 98 F., pulse 90, respiration 20. The thyroid at this time was distinctly nodular and apparently adherent to adjacent structures. A small nodule was made out just above the clavicle on the left side. Malignancy was suspected at this time.

Nov. 14, 1911. Syrup of ferrous iodid was begun in doses of 8 minims t. i. d. The condition of the patient remained but little changed until November 25 when Dr. Hamann removed the left lobe, isthmus and part of the right lobe.

*Pathological Report.*—Right lobe and isthmial portion: The mass is hard, slightly nodular and covered with numerous tags of tissue from the separation of fibrous adhesions. It has neither the shape nor the general appearance of thyroid. Weight of mass 76 grams. On section the surface nodules are found to be adenomatous masses five in number, the largest measuring 3 by 3 cm. One contains calcareous deposits, another is cystic, and the smaller ones are glandular throughout. These adenomas are imbedded in a hard, white scar tissue throughout which are yellowish, necrotic areas varying in size from 1 mm to 6-8 mm. These areas resemble the caseation seen in certain types of tuberculosis or actinomycosis. No true thyroid parenchyma is made out. The general appearance is that of a chronic inflammatory process in a pre-existing adenomatous goiter.

*Microscopic Examination.*—Sections from seven areas of the tumor mass were studied. In only one was there evidence of non-tumor thyroid tissue and this was very sclerotic, with small, shrunken follicles. Sections through two of the adenomas revealed characteristic adenomatous structure composed of small, uniform, closely-set colloid-containing follicles in the subcapsular zone with atrophy and hyaline degeneration of the central portion. The non-adenomatous tissue is for the most part dense, infiltrated fibrous tissue with scattered islands of tumor cells occupying clefts and quite frequently filling the lumina or veins. These tumor areas correspond to the necrotic yellowish areas noted in the gross specimen. The cells are uniformly distributed throughout a given tumor area with no differentiation into follicle formations. The cells are highly irregular in size. Their nuclei are irregular in size and staining intensity, resembling sarcoma as much as carcinoma. Hemorrhage and necrosis are present in all the larger areas. In a few of the areas suggestive follicle formations are present owing to the presence of very scant stroma. On this account the possibility of the tumor being one of those rare, sarcocarcinomas comparable to those described by Wells[4] in the dog, by Leo Loeb[5] in the rat, and Woolley[6] in man, was considered. Almost all of the described cases of this double tumor have been primary thyroid tumors. A study of the metastases in our case eliminates this possibility.

Dec. 1, 1911. Wound healing, back rest, temperature 98 to 99 F., pulse 90 to 100, respiration 18 to 20.

Dec. 8, 1911. Sodium iodid grs. 3 t. i. d. substituted for the syrup of ferrous iodid. Wound healed, temperature normal, pulse 80 to 90, respirations 20.

Dec. 18, 1911. Patient complains of pain in the right thigh and knee. A small mass on the inner side of the right ilium was made out. *X*-ray plates at this time were negative.

Dec. 23, 1911. Patient's general condition fair. Was discharged.

Jan. 2, 1912. Patient re-admitted complaining of pain in the region of the eighth rib in the right axillary line and in the right hip. Physical examination shows no noteworthy change in the neck region. There is a small palpable tumor mass attached to the eighth rib in the axillary line and a tender mass in the right inguinal region. The face had a sallow, puffy appearance. There is increased dulness over the manubrium. The superficial veins of the thorax and abdomen are becoming prominent and somewhat tortuous. An *x*-ray plate taken at this time shows mediastinal growth extending to the left and to the right of the base of the heart; also a mass in the left ninth rib posteriorly.

Jan. 11, 1912. Complains of pain in the right inguinal region and in the right arm. The face is cyanotic and edematous; veins of the neck very much distended.

Jan. 15, 1912. Patient is quite ill; face greatly enlarged and cyanotic. The temporal veins are much distended. Motor power on the right side is decreased. Reflexes diminished. Right arm swollen.

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[4] Wells, H. C.: Jour. Pathol. and Bacteriol., 1901, vii, 357.

[5] Loeb, Leo: Am. Jour. Med. Sc., 1903, N. S. cxxv, 243.

[6] Woolley, P. C.: Am. Med., 1902, iv, 331.

Jan. 16, 1912. Tongue enlarged. Speech and swallowing difficult. Morphin grs. ¼ two to three times per day necessary to control pain.

Feb. 24, 1912. The mass in the right inguinal region had progressively enlarged. Superficial veins of the abdomen and thorax have undergone further enlargement and edema of the face, tongue and neck region is very much lessened.

March 16, 1912. Patient had lost much weight, most marked pain now is in the thorax. Well developed collateral circulation around the obstructed superior vena cava.

April 9, 1912. Obstinate constipation, great emaciation, continuous pain, anxious pinched appearance, catheterization necessary.

April 19, 1912. Patient died this morning. Autopsy three hours later.

*Autopsy Report.*—Body length 170 cm. Marked emaciation; rigor mortis present.

Old thyroidectomy wound. The laryngeal cartilages and neck muscles are obscured by diffuse subcutaneous thickening. There is marked tortuosity of the veins of the chest and abdomen. A large rounded mass about 12 cm. in diameter in the right iliac fossa. The right leg muscles are distinctly atrophic.

Internal: On removing the sternum the tumor mass was found to involve the manubrium and both clavicles. No excess of fluid in the pleural cavities. The thymus area is replaced by tumor tissue which is continuous with the thyroid area above. Right lung free from adhesions and air-containing. Many nodules varying from .1 to 5 cm. in diameter are scattered throughout the lung. No consolidation. Bronchi reddened and contain some mucopurulent exudate. Left Lung: Few adhesions at apex and near the base, air-containing. Multiple tumor nodules of approximately the same size and distribution as in the right lung. These tumor nodules are grayish opaque in color. Many of the masses have central areas of necrosis. In the eighth rib on the right side there is a fusiform swelling which on section proved to be a medullary metastasis. On the left side springing from the ninth rib is a hemispherical mass 4 by 5 cm. in diameter projecting into the pleural cavity but completely covered with pleura.

Heart: Pericardial surfaces smooth. There is no excess of fluid. Heart muscle soft and flabby. The superior cava is completely blocked by tumor growth, distending the vessel to a diameter of 2 to 2.5 cm. and extending up into the innominates and down into the right auricle. Pulmonary veins, inferior cava, aorta, and pulmonary artery free. Liver: Normal appearance, free from metastasis. Spleen: Slightly enlarged. Malpighian bodies and trabeculae prominent. Kidneys: Right measures 11 by 6.5 by 4.5 cm. Capsule strips readily. On the surface are many yellowish raised spots distributed over the entire cortex. On section the surface spots extend through the cortex as yellow streaks. Left kidney similar in all respects to the right. Adrenals normal. Pancreas normal. There is a small metastasis in the mesentery of the transverse colon. Occupying the right iliac fossa and elevating the iliacus muscle and crural nerves, also extending downward into the pelvis and involving the rectum, is a large mass about 15 cm. in diameter. The mass extends through the ilium, involved the ischium at the acetabulum, and also the right side of the sacrum. On section the tumor tissue is soft, grayish, hemorrhagic, with extensive areas of necrosis. There is a separate metastasis in the body of the fifth lumbar vertebra. The thyroid area was removed with the tongue. The left lobe and isthmus are operatively absent. The entire area was now filled with scar tissue and tumor growth. The right lobe area contains a tumor mass in which no distinct thyroid tissue is visible. The esophagus is free save for very prominent varices in the upper and lower thirds. Several small tumor masses project into the lumen of the trachea between the cricoid cartilage and the bifurcation.

*Microscopic Examination.*—Pancreas and liver normal. Spleen: Malpighian bodies and pulp cells well preserved. Kidney: No increase in connective tissue. Throughout the cortex are irregular areas of cellular exudate composed for the most part of polymorphonuclear leukocytes. In some of the areas the tubules remained visible while in others actual abscess formation has taken place. Throughout all the acute inflammatory areas the tubules contain pus cells. Diagnosis: Acute suppurative nephritis. Heart muscle normal. Lung: Congested; moderate coal pigmentation; free from inflammatory exudate.

Thyroid Area—Right Lobe: Four sections. No trace of thyroid tissue visible. Extensive areas of tumor cells in the dense stroma. The tumor masses for the most part show no follicle formation. In some, however, slight differentiation but without formation of lumina can be made out. Same irregularities in the cell types as were noted in the operative specimen.

Lung Metastases: Slight encapsulation; central areas necrotic. The cortical zone is divided into small columns and strands of tumor cells by a very delicate vascular stroma. Slight evidence of follicle formation. The inguinal tumor mass also exhibits slight follicle formation. Rib metastasis: Fibrous stroma more prominent and distinct follicle formations can be made out, some of which contain colloid-like material. The tissue is distinctly thyroid in type although the cells are as atypical as in other metastases, with less evidence of follicle formation. The bronchial lymph-glands contain tumor metastases, the cells of which showed no follicle formation. Tumor from superior cava contain a delicate capillary stroma in places outlining very irregular attempts at follicle formation. The cell types are highly irregular. Nuclear figures are present.

*Anatomical Diagnosis.*—Adenocarcinoma of the thyroid, medullary type. Metastases into trachea, the mediastinum, bronchial lymph-glands, lungs, ribs, fifth lumbar vertebra and ilium. Acute suppurative nephritis.

Iodin determinations were made on several of the tissues and metastatic tumor nodules obtained at post-mortem as well as on the tumor mass removed at operation.

*Iodin Determinations.*—Operative Specimen No. 1. The scar-like tissue corresponding to the thyroid area contains 0.02 mg. per gram of dried tissue.

No. 2.—Adenoma freed from all other tissues contains 0.17 mg. per gram of dried tissue.

Autopsy Tissues:

No. 1.—Liver: Iodin per gram dried tissue, 0.00 mg., or a trace.
No. 2.—Kidney: Iodin per gram dried tissue, 0.01 mg.
No. 3.—Pancreas: Iodin per gram dried tissue, 0.03 mg.
No. 4.—Spleen: Iodin per gram dried tissue, 0.00 mg.
No. 5.—Lung: Iodin per gram dried tissue, 0.00 or a trace.
No. 6.—Lung Metastasis: Iodin per gram dried tissue, 0.00 mg.
No. 7.—Rib Metastasis: Iodin per gram dried tissue, 0.00 mg.
No. 8.—Iliac Metastasis: Iodin per gram dried tissue, 0.00 mg.
No. 9.—Tumor from the site of the right lobe: Iodin per gram of dried tissue, 0.00 mg.

Iodin Administration: Nov. 14 to Dec. 8, 1911, syrup of ferrous iodid 34 c.c. December 8 to 20, sodium iodid 12 gm. January 8 to April 15, 1912, syrup of ferrous iodid 181 c.c. Total iodin before operation, 17 c.c. syrup ferrous iodid. Total iodin after operation, 12 gm. of sodium iodid and 198 c.c. of syrup ferrous iodid.

CASE 2.—S. R., City Hospital No. 11,167; admitted Sept. 11, 1911. Female, Hungarian, aged 39. Complaint, cough with loss of weight for the past ten months. Past history not obtained.

*Physical Examination.*—Fair development, poorly nourished and cachectic appearance. There is a unilateral tumor mass 5 cm. in diameter in the region of the left lateral lobe of the thyroid. Patient has noticed this enlargement for seven years. Right lobe slightly enlarged but with normal outline. The chest is long and the costal angle narrowed. There is distinct dulness over the right apex and bronchial breathing over both apex and base. Heart, abdomen, and extremities negative. No blood examination. Trace of albumin first noticed December 23, which continued until death. Repeated examinations of the sputum for tubercle bacilli negative until April 30, 1912, although patient had afternoon temperature of 101 to 102.8 F. with pulse averaging 100 to 110.

Dec. 3, 1911. General condition of patient about the same as at admission. The thyroid tumor had enlarged slightly. It is quite hard and not painful. Displacement of the larynx to which the tumor mass is firmly attached. Skin movable over the tumor. Several isolated nodules are palpable in the left supraclavicular fossa. There is distinct dulness over the left apex of the lung and *x*-ray plates showed rather dense shadows in both apices.

Dec. 12, 1911. Evidence of tracheal stenosis and operation advised.

Dec. 20, 1911. The tumor mass was removed.

*Pathological Report.*—The mass consists of the left lobe and isthmus. It is firm, nodular, and for the most part encapsulated but in places the tumor tissue extends through. Two of the superficial veins are filled with tumor tissue. On section the tumor mass is whitish, opaque, with a few small necrotic areas. The tumor is sharply defined from the non-tumor thyroid tissue which has all the appearances of normal thyroid tissue. Coarse fibrous strands cut the tumor mass into lobules. In places colloid-like substance is seen.

*Microscopic Examination.*—The non-tumor thyroid tissue is uniformly colloid-containing, slightly stretched by the growing tumor but in all respects resembles pure colloid thyroid. The tumor is for the most part undifferentiated into follicles. The interior portion is composed of irregular columns of large cuboidal cells closely set and for the most part without evidence of lumina. There are areas throughout this portion of the tumor in which irregular follicle formations occur. In the peripheral parts of the tumor follicle formation is absent. There is a distinct alveolar arrangement dependent on the stroma. The tumor cells are somewhat irregular, elongated, and with their long axes arranged radially in the alveolus. No adenoma-like areas were made out. Diagnosis, medullary carcinomas.

Dec. 24, 1911. Recovery from operation uncomplicated. Temperature 99 to 101 F.; pulse 110 to 115. Sodium iodid grains 5, daily. Patient complains of pain in the right hip.

Jan. 10, 1912. Cachexia and emaciation not improved, breathing is better. The right leg is swollen and somewhat painful. *X*-ray at this time shows metastases in the right side of the sacrum.

Feb. 27, 1912. Syrup of ferrous iodid minims 10 t. i. d. was substituted for sodium iodid. Flatness over the right chest and the infraclavicular region of the left with dulness beneath the manubrium. No recurrent growth in the thyroid region.

March 20, 1912. Patient's condition progressively downward. Respiration 24 to 28, pulse 110 to 113; temperature 99 to 100. Constant pain in the sacro-iliac region.

April 30, 1912. Sputum contains tubercle bacilli. Transferred to tuberculosis sanitarium and died there May 3, 1912.

*Autopsy.*—Autopsy May 5, 1912, after overcoming some difficulties. The body was not embalmed and was in a good state of preservation. Slight discoloration of the abdomen. Examination confined to the chest and abdomen. Thyroid area: Right lobe normal shape, enlarged about twice the normal size. Firm, translucent, and on section contains abundant colloid. A cystic adenoma 2 cm. in diameter is imbedded in this lobe. The isthmus was removed at operation. The left lobe for the most part was removed. There is, however, a mass of tissue 4 by 1.5 cm. occupying the site of the left lobe, and adherent to the surrounding structures. On examination this mass is found to contain both colloid and cancerous thyroid tissue. The thymus area contains three metastatic tumor nodules, the largest being the size of a golf ball. Both lungs are adherent to the parietal pleura. The right lung contains extensive tuberculous caseation and consolidation. Both lungs contain numerous tumor nodules varying in size from a pea to a golf ball. Heart, small; contains no metastases. Abdomen: Liver contains several metastases, one large nodular mass just above the gall-bladder, measures 6 by 5 by 5 cm. Spleen contains one small tumor nodule. Kidneys both contain metastases. Pancreas contains a metastasis 2 cm. in diameter near the duodenum. All metastases observed in the several viscera are grayish opaque in color, soft, friable, and have no gross resemblance to the normal thyroid structure. There are several metastases in the omentum and mesentery. The sacrum contains a large metastasis extending into the pelvis and also producing slight ulceration externally. Stomach and intestines appear normal.

*Microscopic Examination.*—Thyroid: Right lobe has all the appearances of pure colloid thyroid. The small adenoma has a subcapsular zone of well preserved, closely set small follicles most of which contain some colloid. Diagnosis: Intermediate adenoma with involutionary changes imbedded in the pure colloid goiter. Three other sections from the right lobe revealed no metastases. Examination of tissue removed from the region of the left lobe shows for the most part dense scar tissue in which are pockets of tumor tissue without any differentiation into follicles. In another section of this mass considerable non-tumor thyroid tissue similar to that of the right lobe is present. Lung metastases encapsulated. They consist of irregular branching columns of tumor cells with slight follicle formations. The cells for the most part are spindle-shaped with a tendency toward radial arrangement from the center of the column. In some of the columns lumina are made out. Liver metastases are in all respects similar to those of the lungs. Pancreas metastasis is composed of branching columns of spindle-shaped cells. Many of these columns have definite lumina. The pancreatic tissue has undergone extensive post-mortem changes. The kidney metastases have a distinct alveolar arrangement of the tumor cells with little or no tendency to form follicles. The surrounding kidney tissue including capsule, glomeruli and tubular epithelium are relatively normal.

*Iodin Determination.*—Operative Specimen: No. 1.—Iodin per gram of dried non-tumor thyroid, 0.80 mg.

No. 2—Iodin per gram of dried tumor thyroid, 0.03 mg.

Autopsy Tissues:

No. 1.—Right lobe, pure colloid gland, iodin per gram of dried gland, 2.92 mg.
No. 2.—Left lobe, mixed tumor and non-tumor thyroid, iodin per gram of dried gland, 0.16 mg.
No. 3.—Lung metastasis, iodin per gram of dried gland, 0.00 mg.
No. 4.—Liver metastasis, iodin per gram of dried gland, 0.00 mg.
No. 5.—Esophagus tissue, tumor-free, iodin per gram of dried tissue, 0.00 mg.
No. 6.—Pancreas tissue, tumor-free, iodin per gram of dried gland, 0.00 mg.
No. 7.—Liver tissue, tumor-free, iodin per gram of dried gland, 0.00 mg.

*Administration of Iodin.*—Total iodin before operation, 0.

Total iodin after operation: (a) Dec. 24, 1911, to Jan. 5, 1912, 1 gm. sodium iodid daily; total 13 gm. (b) Feb. 27, 1912, to April 25, 1912, 10 minims syrup ferrous iodid t. i. d.; total 119 c.c.

In addition to these two human cases one of us (M.) has observed a case of thyroid carcinoma in a dog, and inasmuch as the data are in all respects similar to those for the human cases it may also be included here. The following is a synopsis of the case record:

Case 3.—Laboratory No. A-124, male Boston Bull-Terrier, aged 8 years, owned by Dr. E. P. Carter, admitted May 1, 1908.

*History.*—Goiter had been known clinically for over three years, with progressive enlargement. First examined dog on Oct. 9, 1907. Thyroid lobes at this time were symmetrically enlarged, compressible, distinct, movable and connected by a broad isthmial band. Diagnosis, active thyroid hyperplasia. Lugol's solution 0.1 c.c. daily was given. This treatment was continued with very slight interruptions until May 8, 1908. The dog was seen from time to time and the thyroid lobes, which at first became smaller, began to enlarge again in February, 1908. Their outlines became less distinct. Gradually both lobes and isthmus became fused into a single mass and the entire mass became firmly adherent to the larynx. In April the thyroid mass was more distorted, firm, and immovable, and was producing dyspnea. As the dog was now losing weight and the thyroid lobes had undergone marked changes, the probability of thyroid carcinoma was suggested and operation advised. May 8, 1908, under ether anesthesia the entire left lobe was removed. It was densely adherent to the trachea, larynx, esophagus and adjacent muscles, and weighed 145 gm.

*Pathological Examination.*—The lobe was nodular, although everywhere encapsulated. Consistency was firm. On section there were still some very definite areas of original thyroid tissue in the colloid state, for the most part lying just beneath the capsule, but in general the entire lobe was replaced by a new growth. The tumor tissue was grayish opaque in color, containing many small hemorrhages and small areas of necrosis, together with a few cystic areas filled with a brownish cholesterin-containing fluid. It was impossible to separate the tumor from the non-tumor thyroid tissue and duplicate iodin determinations were made on the mixed tissue with results of 0.32 and 0.30 mg. per gram of dried gland.

May 11, 1908, dog doing nicely.

May 16, 1908, wound slightly infected, and as the dog had undoubted carcinoma the owner desired him chloroformed.

*Autopsy.*—Slight wound infection. The right lobe weighed 125 gm., was nodular, hard, gray-red, and on section was equally involved with cancer as the lebt lobe, which was removed at operation. Extension of carcinomatous tissue into the anterior mediastinum. Both lungs contained numerous metastatic nodules varying from 1 mm. to 2 cm. in diameter. They were gray-white, friable, and contained no visible colloid. The lymph-glands along the bronchi were involved. No metastases were found in the kidneys, liver, pancreas, or other viscera. As the dog had been getting iodin up to the time of operation, iodin determinations were made on the autopsy tissues as follows:

Right lobe of the thyroid contained 0.27 mg. per gram of dried gland.
Lung metastasis contained 0.11 mg. per gram of dried tissue.
Lung tissue contained 0.07 mg. per gram of dried tissue.
Liver tissue contained 0.15 mg. per gram of dried tissue.

*Histological Examination.*—Thyroid: The capsule was markedly thickened. Slight round-celled infiltration. There were many areas of original non-tumor thyroid containing normal colloid and normal cuboidal epithelium. The tumor tissue was in general differentiated into follicles. These were small, rounded, closely packed and resembled the fetal adenoma follicles seen in human goiter. There were other areas or irregular islands making up probably a third of the sectioned area in which little or no differentiation into follicles could be made out. The epithelium of the formed follicles was high cuboidal in type and the nuclei were large vesicular, quite regular in size and staining intensity. Lung Metastases: Six were examined, all showing some differentiation into follicles, of the same characteristics present in the thyroid lobes.

## SUMMARY AND DISCUSSION

The principle data of Case 1 are: Patient was under observation 168 days—23 days before and 145 days after operative removal of part of the thyroid tumor. Metastases were without doubt present before the patient came under our observation. The thyroid contained several adenomas of the fetal type and while it is impossible to state from what portion of the thyroid the carcinoma originated, we have come to the conclusion from the tumor pattern that it probably originated from an adenoma. The whole course of the disease was, as far as could be determined, one of uncomplicated thyroid cancer. Seventeen c.c. of syrup of ferrous iodid was given before operation and 12 gm. of sodium iodid, and 198 c.c. of the syrup of ferrous iodid were given after operation—iodin having been continuously administered from Nov. 14, 1911, to April 15, 1912, with the exception of eighteen days from Dec. 20, 1911.

Case 2 was in the hospital continuously from Sept. 11, 1911, until her death, May 3, 1912—235 days. She was admitted for and had active pulmonary tuberculosis. Thyroid carcinoma was not definitely recognized until December 3, when, owing to tracheal stenosis, a palliative operation was advised. The tuberculosis doubtless hastened death. The tumor though metastasizing was of slower growth than that of Case 1. The carcinoma and the tuberculosis were joint factors and the individual importance of each cannot be estimated. In this case also there was one typical adenoma in the right lobe and the general type of tumor led us to look on this carcinoma as also originating in an adenoma. No iodin was given prior to the operation. Following the operation iodin was given continuously until April 25, 1912—eight days before death—with the exception of fifty-two days following Jan. 5, 1912. She received a total of 13 gm. of sodium iodid and 119 c.c. of syrup of ferrous iodid.

Case 3 (a dog) was under observation for approximately seven months. Carcinoma was suspected after the fifth month. Small doses of Lugol's solution were administered by mouth with slight interruptions from October until May 8. The total amount of iodin used could not be obtained.

The scar-like mass of the left lobe removed at operation in Case 1, after freeing it from all visible adenomatous tissue, contained 0.02 mg. of iodin per gram of dried tissue. The adenoma from the same lobe contained 0.17 mg. of iodin per gram of dried tissue. The patient had been getting iodin for eleven days before operation. Adenomas of histological differentiation present in this case normally contained some iodin, and while the comparatively high content may be explained on the basis of the iodin administered, this does not seem likely, judging from our experience with adenomas in general. The iodin content of the scirrhus cancer tissue is, on the other hand, probably due to the administration of iodin, judging by the distribution of iodin in the tissues and its rate of excretion following its administration in dogs and rabbits.[7] The iodin contents of the tissues at autopsy, notwithstanding the fact that iodin was given until five days before death, shows no traces of iodin in the remaining scar-like cancerous mass of the right lobe, in the spleen, in the rib, lung, or iliac metastases. Definite traces of iodin were found in the lung and liver tissues while the kidney and pancreas con-

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7. Boruttau: Ztschr. f. Exper. Path. u. Therap., 1910, viii, 418.

tained 0.01 and 0.03 mg. per gram of dried tissue, respectively. The fact that the pure cancer tissues were free from iodin while the adenomas had over eight times as much iodin as any of the other thyroid tissues and about six times as much as the pancreas, possibly suggests that this was the best preserved thyroid tissue. Certainly the carcinoma tissues showed no power whatever to retain iodin, although the tissues were exposed to the most favorable influences for iodin storage.

In Case 2 the portion of the left lobe removed at operation was separated into tumor and non-tumor tissues. The non-tumor tissue contained 0.80 mg. of iodin per gram of dried tissue, while the adjacent tumor tissue contained 0.03 mg. of iodin per gram of dried tissue. This specimen looked like pure cancer tissue, and histological examination of several areas of it showed only tumor tissue. No iodin had been administered for nearly three and a half months. The remaining portion of the left lobe obtained at autopsy contained both tumor and non-tumor thyroid tissue, but was so intermingled that no separation was possible. The mixed specimen contained 0.16 mg. of iodin per gram of dried tissue. The right lobe, which on gross and histological examination was pure colloid thyroid, contained 2.92 mg. of iodin per gram of dried gland, or nearly four times the iodin content of the non-tumor thyroid of the left lobe obtained at operation before iodin was given. In this case neither the metastases in the liver and lung nor the non-tumor liver, kidney, pancreas, lung and esophagus tissues contained any trace of iodin although iodin had been given for a long time and up to eight days before death. This case has the advantage over Case 1 in possessing an abundance of thyroid tissue unaffected by tumor, and it is noted that this tissue reacts with iodin in the characteristically normal physiological manner.

In Case 3 duplicate determinations on the operative specimen showed 0.32 and 0.30 mg. of iodin per gram of dried tissue, respectively. The examined specimens were mixed tumor and non-tumor as the tumor growth had so infiltrated the whole lobe that separation was impossible. The observation is therefore useless so far as determining whether the tumor tissue contained iodin. Likewise, the left lobe removed at autopsy was so infiltrated with cancer that separation was impossible and the iodin determination on a mixed specimen give 0.27. The lung metastases showed 0.11 mg. of iodin per gram of dried tissue, while the tumor-free lung and liver—the only other tissues examined—gave 0.07 and 0.15 mg. of iodin per gram of dried tissue, respectively. From these findings and from the general distribution of iodin in the body following its administration it is probable that all the tissues contained iodin. The fact that the iodin content of the liver was higher than that of the lung metastasis renders the iodin content of the lung metastasis of no physiological significance, and the iodin content of the lung and liver is probably due to the non-excretion of the recently administered iodin. Whether iodin in the form of Lugol's solution is held by the tissues longer than iodin given as syrup of ferrous iodid is a question barely suggested by the striking difference in the iodin contents between the two human cases and the dog. In most of the work on the retention of iodin in the tissues potassium and sodium iodid have been used, the last traces of which are known to be excreted slowly. The administration of iodin in these three cases had neither a detectable inhibiting influence nor a detectable accelerating influence on the rate of growth.

In these three cases, therefore, it seems that cancer tissue exhibits practically no capacity for iodin retention, contrasted with the non-tumor thyroid, which has a marked capacity for storing iodin; and with benign epithelial tumors (adenomas), all of which in some phase of their existence have some capacity for iodin, thyroid cancer exhibits the least—in our three cases probably no capacity for iodin. We have been able to find but two incomplete reports in the literature of iodin determinations in metastatic thyroid carcinoma. Ewald[8] reported one case in which the examination of the primary tumor (a thyroid adenoma) showed no iodin, while metastases in the lung and lymph-glands showed appreciable amounts. No other details of the case are given. He suggested that further examinations should be made, since if Baumann's observations and conclusions should be established the iodin contents of tumors might afford evidence as to tumor function. Gierke[9] observed a vertebral tumor with thyroid-like structure which contained approximately 0.25 mg. of iodin per gram of dried tissue. No other details are given and on this account it is impossible to make comparisons with our tumor cases. As there are more differentiated types of thyroid carcinoma, or possibly more correctly stated, there are metastatic thyroid growths especially in bone which morphologically closely resemble fully differentiated thyroid tissue, the need of more extended observations is obvious, as it might be possible to determine through their iodin function what relation they bear to tumors, since morphology is of little value. As dogs are not known to have adenomas we suppose that the carcinoma in Case 3 arose from tissue that had undergone physiological differentiation. If such was the case the physiological attributes have been lost, as the tumor behaved towards iodin in a similar manner to the two human carcinomas. We have as yet no direct evidence that thyroid tumors, whether benign or malignant, may compensate for the function of the non-tumor thyroid. Experimental approach has so far been impossible because none of the lower mammals is known to have true adenomas. The often quoted observation of von Eiselsberg[10] to the effect that following the removal of a large goiter tetany developed which cleared up as sternal thyroid metastases developed, was made at a time when parathyroid and thyroid functions were not clearly separated. Such symptoms are not now believed to have connection with thyroid. More applicable are the observations of Gulliver[11] where a woman 44 years old with symptoms of myxedema dating back four years developed thyroid carcinoma with metastases, but without any noteworthy amelioration of the myxedema symptoms. This author also cites an observation reported by Hilton Fagge where a sporadic cretin with a large goiter developed thyroid carcinoma without any improvement in the cretinoid condition. Such clinical observations though meager are in harmony with the experimental chemical evidence of the cases here reported, viz., that in thyroid carcinoma there is no evidence that useful function is preserved, but rather that in becoming malignant the tissue loses most of its physiological attributes. So far as observations have extended, it would appear that benign epithelial tumors occupy an intermediate position as regards evidence of function between fully differentiated thyroid tissue on the one hand and malignant epithelium tumors on the other.

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[8] Ewald: Ueber den Jodgehalt des Adenocarcinome der Schilddrüse und seine Metastasen. Wien. klin. Wchnschr., 1896. lx, 186.

[9] Gierke: Ueber den Jodgehalt von Knochentumoren mit Schilddrüsenbau. Beitr. z. chem. Physiol. u. Path., 1903, iii, 286.

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[10] Von Eiselsberg: Arch. f. klin. Chir., 1894, xlviii, 489.

[11] Gulliver: Malignant Disease of the Thyroid Developing in a Case of Myxedema. Tr. Path. Soc., London 1886, xxxvii, 511.

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# BENIGN EPITHELIAL TUMORS OF THE THYROID GLAND.*

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## I.

INTRODUCTION. — Among the most interesting and certainly the least understood of the thyroid tissue reactions is the development of benign epithelial tumors — the so-called adenomas.

It is the purpose of this paper to review the morphological characteristics of these tumors; to present our observations on the percentage iodin contents in relation to their anatomical structure; to offer a classification that embodies both the morphological and physiological data and, lastly, to discuss the possible bearing of these results on Cohnheim's hypothesis of tumor origin.

CLASSIFICATION. — The accumulation of this material has extended over a period of several years, for the reasons (1) that human material only can be used and (2) that these tumors as we see them at autopsy or operation so frequently represent terminal conditions as cysts, fibroid or calcified masses that pure types are relatively rare. Thus out of a total of six hundred and seventeen pathological specimens of human thyroid one hundred and forty-one consisted of or contained benign epithelial tumors and of these only ninety-three were sufficiently free from complications or destructive changes to be used. Many of these

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ninety-three specimens were slightly complicated either by hemorrhage, mild cyst formation, calcification or fibrosis, but sufficient tumor tissue was preserved uninjured for purposes of study and classification. Several of these ninety-three cases were tumors accidentally found at autopsy or in specimens removed for other causes as exophthalmic goiter or simple goiter.

By thyroid adenoma one ordinarily means a circumscribed, localized, and encapsulated growth of glandular tissue which retains more or less of the anatomical characteristics of the parent tissue, although the term was formerly used by Rokitansky,[1] Virchow,[2] and Wölfler[3] to include symmetrical hyperplasias of the whole gland (*e.g.*, " colloid adenoma "). While at present there is a tendency to sharply distinguish morphologically between adenomas and diffuse overgrowths of the thyroid (goiter) and to criticise the earlier observers' use of the term " adenoma ; " nevertheless, the more extensive one's acquaintance with these growths becomes the more one is convinced that the point of the earlier writers was well taken; that there is not the sharp division between benign epithelial tumors and diffuse overgrowth that some have been led to expect either anatomically or functionally. There are all gradations, anatomically, from the one to the other and functionally it is known that the development of these benign tumors is constantly associated with hyperplasia of the physiologically adult thyroid tissue, or that the development of these tumors is a part of a general physiological disturbance which causes the gland as a whole to enlarge.

The experimental demonstration of a physiological cycle characterized by developmental, involutionary and colloid or resting phases was a most important aid in the sequential grouping and classification of the almost innumerable degrees of progressive and regressive changes in the hyperplasias of the fully differentiated thyroid tissue.

This cycle of cell changes can easily be reproduced experimentally and the progressive or developmental phase was first clearly demonstrated by Halsted[4] in his classical experiments with partial extirpation. In 1909[5] we were able to demonstrate experimentally one of the regressive phases — involution — by showing that the experimental compensatory hyperplasia of Halsted began to involute spontaneously as soon as the physiological compensation was complete, and further that this involutionary process in dogs could be hastened by the use of iodin. These facts have also been demonstrated in spontaneous thyroid overgrowth (goiter). A second form of regressive change — atrophy — is frequently demonstrated in spontaneously occurring myxedema.

So that in interpreting the thyroid changes in goiter one must bear in mind this simple thyroid cycle which, beginning with the normal cell, consists of progressive changes through all degrees of hypertrophy and hyperplasia and regressive changes either (1) throughout all degrees of atrophy, or (2) throughout all degrees of involution to the resting or colloid or nearest normal state that such thyroids can again assume which have once undergone hyperplasia.

The cycle may be represented schematically as follows:

Normal thyroid—hypertrophy—hyperplasia { atrophy
{ involution to colloid state

Colloid state—hypertrophy—hyperplasia { atrophy
{ involution to colloid state

Colloid . . . .

This conception has made it possible to interpret the many gradations of thyroid reactions found in diffuse overgrowths of the thyroid (goiter), and now one looks upon any given specimen of thyroid reaction as a phase of this cycle and not as an entity either anatomically or physiologically.

Virchow,[6] from his acquaintance with these tumors, recognized three anatomical groups depending on the predominance of (1) gland follicles, (2) stroma, (3) blood vessels.

This was followed by Wölfler's classical studies,[3] and his classification has been the basis for all subsequent attempts.

Both Virchow and Wölfler were unable to solve the problem of deciding what enlargements were to be considered as tumors and what were merely diffuse overgrowths of physiologically adult tissue. This difficulty, as will be shown later, can now be partially overcome by studying the effect of iodin on these tumors during life. He recognized the following groups :

- (1) adenoma fetalis
  - acinosum
  - fibrosum
  - vasculosum
  - cavernosum
  - papilliforme
- (2) adenoma gelatinosum
  - interacinosum
  - cysticum
- (3) adenoma myxomatosum
- (4) adenoma cylindrocellulare

While Wölfler was familiar with all the morphological characteristics of these tumors and gives excellent discussions as to their modes of origin and terminations one cannot agree that fetal adenoma and myxomatous adenoma are of the same value as basic types or that colloid adenoma is a form of degeneration. The term "colloid adenoma" is not used to-day as synonymous with colloid goiter. Neither is it proper to designate a colloid goiter composed merely of enlarged follicles as "cystic adenoma" or "cystic goiter."

Confining ourselves for the moment to the histological structure of relatively uncomplicated tumors, it has long been known that certain broad groups could be made. Thus the "fetal adenoma group" has been a well recognized morphological group since the studies of Stromeyer,[7] B. Beck,[8] Joh. Müller,[9] and Billroth.[10] Then there is a more or less defined group of "simple adenomas" (colloid adenomas of Wölfler) so called on account of their close resemblance in structure (epithelium, colloid, size of follicles, etc.) to the simple diffuse hyperplasia — differing from it largely in being encapsulated. Between the fetal adenomas on the one hand

and simple adenomas on the other, most observers have noted intermediate forms.

In our material these general groups could be distinguished or better, perhaps, the series showed all gradations from the type closely resembling simple thyroid hyperplasia to the true fetal adenoma type. Adding to this series of morphological gradations dependent on the anlagen from which the tumors develop the conception that the tumor developing from each anlage undergoes its cycle of cell changes (progressive and regressive) and it can be seen that the morphology of these tumors may vary both with the type of cell from which the tumor took origin and also with the phase of the physiological cycle of the tumor cells at the time of the examination.

This being true it is clear that studies in morphology alone could not estimate the significance of morphological variations dependent upon physiological activity.

We therefore attempted to ascertain whether anything comparable to the physiological cycle of the non-tumor tissue could be made out in the tumor tissue by comparing the iodin contents of these tumors with their histological structure and studying the effect of the administration of iodin to patients with these tumors. The results will be referred to again in detail and it suffices here to state that the percentage iodin content was found to bear a relation to the structure — being lowest in the growing and highest in the resting or colloid phases, and that the administration of iodin affects these tumors to some extent — the effect being least in the fetal adenomas and greatest in the simple adenomas.

These observations suggest that these tumors have a physiological cycle of cell changes consisting of a growing, involutionary and colloid or resting phases much modified but in general comparable to that which occurs in the fully differentiated thyroid tissue and if represented schematically would be:

$$\left.\begin{array}{c}\text{Adenoma}\\\text{anlage}\end{array}\right\}\text{hypertrophy—hyperplasia}\left\{\begin{array}{l}\text{atrophy}\\\text{destructive metamorphosis}\\\text{involution to colloid or resting state.}\end{array}\right.$$

In the light, therefore, of definite morphological evidence of variation in the type of tumor depending on the degree of differentiation possessed by the cells of the tumor anlage and of definite physiological evidence of variation in structure, depending on the functional phase (progressive or regressive) of the tumor, we would submit the following scheme of classification :

THYROID GLAND.

| I. | II. | III. | IV. |
|---|---|---|---|
| Hyperplasia from Physiologically Differentiated Thyroid (Simple or Parenchymatous Goiter). | Simple Adenoma. | Intermediate Adenoma. | Fetal Adenoma. |
| (1.) Growing phase. | Growing phase. | Growing phase. | Growing phase. |
| (2.) Involutionary phase. | Involutionary phase. | Involutionary phase. | Involutionary phase. |
| (3.) Colloid phase. | Colloid phase. | Colloid phase. | Colloid phase. |

We have divided the benign epithelial overgrowths of the thyroid tissue into four primary groups, using as fixed points the simple hyperplasias (ordinary goiter) or the most differentiated of all thyroid hyperplasias as one end of the series and the fetal adenoma group or the least differentiated of benign thyroid hyperplasias as the other end. Between these two fixed and constant groups one encounters all gradations which for the sake of simplicity and convenience of description we have divided into two only, — simple and intermediate adenomas. We have also divided the physiological cycle of cell changes of each of the four primary groups into three phases, using as our fixed points the growing stage and the colloid or resting stage. Between these two relatively constant there are all intermediate stages which, for the sake of simplicity, may be collected into one group — involutionary.

Group I. comprises the simple hyperplasias which develop from the most differentiated thyroid tissue. These hyperplasias are in no sense tumors. They are the ordinary thyroid tissue overgrowths seen in all developing goiters in all animals from fish to man. We have introduced this best known group of thyroid tissue overgrowths into the scheme in order to show its relations both anatomical and physiological to the localized and encapsulated epithelial overgrowths.

Group II. comprises the simple adenomas, that is, circumscribed masses of thyroid hyperplasia which in their morphological characteristics and their reactions with iodin closely resemble the ordinary hyperplasias of Group I. These tumor-like masses are never seen in glands which are not also the seat of a general hyperplasia, and in a large series of thyroid examinations one finds all gradations between the simple hyperplasias of Group I. and strictly encapsulated hyperplasias. Many of these masses can be classed as tumors morphologically only in the sense that they have fibrous capsules (often very rudimentary) (see Figure 17). Physiologically they are always affected by iodin, occasionally to the same degree as the non-tumor thyroid tissue. So that areas of encapsulated hyperplasia which morphologically are often classed as simple adenomas physiologically may not be tumors. The dividing line then between simple diffuse hyperplasia and simple adenoma lies somewhere between Groups I. and II. and the exact status of any given mass can only be determined by its reaction with iodin.

Group III. comprises the intermediate adenomas — an arbitrary grouping to include encapsulated masses of epithelial hyperplasia not differentiated sufficiently to be included under Group II. and more differentiated than the fetal adenomas. These growths are undoubtedly tumors, since morphologically they are composed of small rounded follicles sometimes closely and at others loosely placed in a scanty stroma within a well formed fibrous capsule and are not notably influenced by iodin within the time limits in which simple hyperplasia is affected.

Group IV. comprises the true fetal adenomas. Wölfler included under fetal adenoma both our Groups III. and IV.

They are the least differentiated of all benign thyroid epithelial overgrowths and the most perfectly encapsulated. They are also the least affected by iodin and are therefore the most autonomous of the benign epithelial overgrowths.

In each of these four groups there is a well defined cycle of cell changes characterized by a growing, an involuntary, and a colloid or resting stage. This cycle is regular and constant in the simple hyperplasias of Group I. and becomes more irregular and less constant through Groups II., III., and IV. The growing phase is characterized anatomically by an increased vascularity of the tissue, an increase in the size and number of the gland cells, becoming columnar from a previously cuboidal state and a decrease in the stainable colloid. Physiologically, this change is characterized by a decreasing percentage iodin content. The involutionary stage is characterized by a decreasing blood supply, a decrease in the size of the epithelial cells from columnar toward cuboidal and an increase in the stainable colloid. Physiologically there is an increasing percentage iodin content.

In the colloid stage the blood supply, the epithelium, the stainable colloid and the percentage iodin content approach and may coincide with the strictly normal gland tissue. This physiological cycle though present throughout the tumor groups is progressively less pronounced morphologically and physiologically as one passes from the simple hyperplasias to the simple adenomas, to the intermediate adenomas, and is least pronounced in all its characteristics in the fetal adenomas.

## II.

## THE RELATION OF THE PERCENTAGE IODIN CONTENTS TO THE ANATOMICAL STRUCTURE AND TO THE PHYSIOLOGICAL PHASES OF THESE TUMORS.

Of the ninety-three tumor masses used in this study, iodin determinations were made on eighty-seven, of which thirty-six were grouped, anatomically, under fetal adenomas;

twenty-five under intermediate adenomas, and twenty-six under simple adenomas.

We were fortunate in obtaining sufficient non-tumor thyroid tissue in fifty-six of the ninety-three cases to make histological studies and iodin determinations. Of these fifty-six, twenty-five were from cases in the fetal adenoma group, fourteen from cases in the intermediate adenoma group, and seventeen from cases in the simple adenoma group.

In analyzing these iodin determinations one of the most important factors to have accurate knowledge of would be whether iodin had been used in the treatment or not. The tumor material being of necessity all human, it is impossible to obtain this knowledge for all cases and we will therefore discuss separately those cases which were given iodin either to our personal knowledge or from whom very positive statements were obtained. There were twenty-three such cases, of which eight had been given syrup of ferrous iodid for from two weeks to two years (intermittently) under our personal direction. The remaining fifteen had been given several preparations, including potassiumiodid, sodium iodid, hydriodic acid and Lugol's solution.

In one case (H. 454) not included in these twenty-three, desiccated thyroid was given for two weeks before operation. The iodin content of the non-tumor thyroid was .53 and of the tumor .11 milligram iodin per gram dried.

As others in addition to those twenty-three cases may have received iodin in some form at one time or another a detailed analysis loses much of its value. We have, however, made three analytic groups: (1) the eighty-seven cases as a whole, (2) the sixty-four cases which had not taken iodin recently, to our or the patient's knowledge, and (3) the twenty-three cases which to our knowledge had received inorganic iodin.

A factor which modifies the value of the iodin determinations of the non-tumor thyroid tissue is that owing to stretching and atrophy by pressure from the enlarging tumor, its physiological activity is greatly impaired and often completely destroyed when the surrounding non-tumor tissue is reduced to layers of fibrous tissue (false capsule of tumor). This modifying factor has been partially eliminated by excluding all (six) cases where there was general destruction of the follicles histologically.

TABLES OF AVERAGES.

*Iodin per gram of dried gland in milligrams.*

| | Fetal Adenomas. | | Intermediate Adenomas. | | Simple Adenomas. | |
|---|---|---|---|---|---|---|
| | Non-tumor Thyroid. | Adenoma. | Non-tumor Thyroid. | Adenoma. | Non-tumor Thyroid. | Adenoma. |
| No. 1.............. | Of all cases = 87. | | | | | |
| (1) Growing...... | 1.02 | 0.09 | 1.01 | 0.23 | 1.31 | 0.59 |
| (2) Involutionary.. | 0.89 | 0.25 | 1.93 | 0.46 | 0.77 | 0.35 |
| (3) Colloid...... | 1.02 | 0.94 | 0.98 | 0.45 | 1.08 | 0.65 |
| No. 2.............. | Of cases not fed iodin = 64. | | | | | |
| (1) Growing...... | 0.46 | 0.11 | 0.75 | 0.22 | 0.43 | 0.18 |
| (2) Involutionary.. | 0.60 | 0.25 | 0.44 | 0.45 | 0.77 | 0.35 |
| (3) Colloid....... | 0.14 | 0.78 | 0.74 | 0.36 | 0.69 | 0.46 |
| No. 3.............. | Of cases fed iodin = 23. | | | | | |
| (1) Growing...... | 2.02 | 0.06 | 1.28 | 0.32 | 1.75 | 0.80 |
| (2) Involutionary.. | 1.76 | 0.26 | 3.05 | 0.47 | | |
| (3) Colloid....... | 1.47 | 1.05 | 1.93 | 1.03 | 1.95 | 2.22 |

(1.) As can be seen in the complete charts or in the complete iodin tabulations (appended at the end of the paper) or in the tables of averages above, the percentage iodin content of the non-tumor thyroid tissue bears no evident relation to that of the tumor in the growing phase of either of the three groups. Thus the percentage iodin content may be relatively high in the non-tumor tissue and iodin measurably absent from the tumor, or both may be low. The percentage iodin content of the tumor is in most instances far less than that of the non-tumor tissue, although this is not necessarily so, as is easily intelligible when one recalls that in infants' thyroids normally and in marked hyperplasias of physiologically adult tissue the percentage iodin content may be very low or iodin measurably absent.

Another feature of physiological significance is that the growing stage of fetal adenomas has the lowest percentage iodin content, the growing stage of intermediate adenomas the next lowest, and the growing stage of simple adenomas the highest. The growing simple adenomas, therefore, approach most closely the growing simple non-tumor hyperplasias in this capacity for iodin.

Taking up the three functional phases (growing, involutionary, and colloid) as grouped according to structure, the most striking feature is that the growing phases of all three groups have in general distinctly lower percentage iodin contents than the involutionary phases, and the involutionary phases in general have lower percentage iodin contents than the colloid or resting phases. This is quite striking when one considers that the grouping is purely on the basis of structure and that in human material many of the most important aids are lost on account of our ignorance of the life history of the individual tumors. This loss is compensated for in a way by the detailed experimental knowledge of the analogous functional phases of the non-tumor thyroid tissue which undergoes a similar cycle of growing, involutionary, and colloid or resting phases.

(2.) Cases which had not received iodin recently to our or the patient's knowledge: This tabulation differs from the first only in being minus the twenty-three cases known to have taken inorganic iodin. As one could prophesy from previous knowledge of the behavior of the physiologically adult thyroid tissue in the presence of iodin, the greatest difference between the two tabulations occurs in the percentage iodin contents of the non-tumor thyroid tissue which is distinctly lowered, while those of the tumors are but slightly modified. Apart from this feature the same general relations are evident.

(3.) Those cases which, to our knowledge, received iodin: The twenty-three cases in this tabulation are equivalent to experiments. They lack iso-controls so that we can compare them only in a general way with those cases not known to have received iodin recently.

First and most striking is the relatively high iodin content of the non-tumor thyroid tissue which, considering the smallness of the series, is singularly constant through the three groups of adenomas and the three physiological phases of these groups. This finding is only confirmatory of the well known characteristic of thyroid tissue, viz., that a rapid storage of iodin takes place in the thyroid following the administration of iodin.

The next feature of importance is the relative independence of the tumor and the non-tumor tissues as regards ability to take up iodin. This independence is practically complete in the growing phase of the fetal adenomas as judged by comparison of the percentage iodin contents of the same group of tumors in Tabulation 2, but seems to become less marked as one passes to the intermediate and to the simple adenomas. The probable explanation of this is that with the increasing anatomical differentiation there is also increased physiological differentiation (as judged by capacity for iodin). In the simple adenomas it clearly approaches the degree of iodin handling function exhibited by the non-tumor or physiologically adult tissue.

So also the differences in the percentage iodin contents between the tumor and the non-tumor tissue become less as one passes from the growing phases through the involutionary to the colloid or resting phases. In the colloid phases of all three tumor groups the percentage iodin contents closely approach those of the non-tumor tissue and in individual cases may exceed that of the non-tumor tissue as is the case in the colloid phase of the simple adenomas. One sees, therefore, an increased storage of iodin varying directly with the structural differentiation of the tissue anlage from which the tumor started and also varying directly with the physiological phase of the tumor.

Another feature that is suggested in Tabulation 2 and here more clearly illustrated by the iodin reaction is that as the tumor grows (whether from the starting point of fetal, intermediate or simple adenoma) it tends to differentiate functionally as well as morphologically. In other words, the thyroid tissue of these benign tumors tends to and does undergo a modified cycle of cell changes similar to that which the fully differentiated tissue undergoes and differing from it only in degree. This degree depends on the anatomical and physiological differentiation of the anlage from which the tumor takes origin. Thus a tumor starting from the fetal thyroid tissue or the lowest anatomically and physiologically differentiated tissue of our tumor groups would, under the usual conditions obtaining in an animal, undergo a more modified and therefore a more independent cycle than would a tumor starting from a relatively well differentiated anlage as represented in the anlage of a simple adenoma. By the phrase " under the usual conditions obtaining in an animal " we refer to the fact that usually there is an abundance of growing non-tumor thyroid tissue where there is a growing adenoma, but if it were possible to obtain an animal in which these tumors occurred where these conditions could be experimentally altered, as, for example, by removing all the non-tumor thyroid tissue and preserving an adenoma with its blood supply, it is highly probable that such an adenoma, even though it were of the lowest type (fetal), would undergo morphological and physiological differentiation more rapidly than it would do or does in the presence of more highly differentiated thyroid tissue which could have the effect of inhibiting the differentiation of the tumor tissue.

Summing up the evidence obtained from these tabulations of the percentage iodin contents arranged according to structure, one sees that just as the non-tumor thyroid tissue shows a variable anatomical state in association with the several groups of tumors so also the iodin contents are variable. There is, however, in the non-tumor thyroid tissue a definite relation between structure and iodin content just as in the physiological hyperplasias of all mammals and in the tumors a similar relation exists. That is, the percentage iodin contents are lowest in the growing phases and highest in the colloid or resting phases.

There is a physiological cycle for these tumors as determined by the iodin contents just as there is an anatomical cycle as determined by their structure and this physiological cycle, just as the anatomical cycle, differs from the corresponding cycle of the non-tumor tissue only in degree. That is, the tumor tissue is, under natural conditions, not capable of producing as highly differentiated a type of hyperplasia as the non-tumor tissue and its hyperplasia is not under the strict physiological control that the non-tumor thyroid hyperplasia is. Thus simple hyperplasia of differentiated thyroid tissue is constantly and quickly modified by iodin both in structure and iodin content, while the tumor hyperplasia is neither quickly nor constantly modified. In true fetal adenomas there is, as a rule; no evidence of any effect of iodin in the usual time (2–6 weeks), in which non-tumor thyroid tissue is markedly changed as shown in five examples (H. 239, 240, 242, 348, and 359), in which inorganic iodin was used. In each of these cases the surrounding non-tumor tissue was markedly affected both in structure and in iodin content.

As one passes from these least differentiated (fetal) adenomas to the more differentiated intermediate and to the still more differentiated simple adenomas, there is evidence of increased iodin action both on structure and on iodin content, as shown in Nos. H. 447, 508, 526, and 555, examples of growing simple adenomas fed iodin.

Between the simple hyperplasias and the simple adenomas there are intermediate gradations both in structure and in the action of iodin, and by means of their iodin reactions it is possible to state that there are certain masses of epithelial overgrowth with the physical characteristics of benign tumors which are physiologically not tumors but parts of the general functional overgrowth of the gland which in growing had acquired a kind of encapsulation. In other words, the diagnosis of benign thyroid tumors on the basis of anatomical structure leads to error unless controlled and checked by some physiological test, just as the overgrowth of the thyroid in bony fish exhibits certain anatomical peculiarities which by purely anatomical standards we would recognize as malignant epithelial infiltration while by physiological tests they belong to clearly benign physiological overgrowths.

Finally the question arises: Do these benign tumors functionate? From all evidence at present available we must conclude that they have function. In the first place these tumors are never seen in thyroids which are not also or have not been the seat of a general hypertrophy or hyperplasia. Secondly, one must recognize a complete gradation of these tumors anatomically from the least differentiated or fetal type through the intermediate and simple adenomas to the strictly physiological hyperplasias of physiologically adult or highest type of thyroid tissue. Thirdly, one finds all gradations in the percentage iodin contents from the lowest (occasionally measurably absent) in the fetal adenomas through the intermediate and simple adenomas to the highest in the physiologically adult hyperplasias. Fourthly, the administration of iodin does influence these tumors. This influence is least (ordinarily unrecognizable in 2–6 weeks) in the fetal adenomas, then gradually increases through the intermediate and the simple adenomas and reaches its fullest physiological effect in the simple diffuse hyperplasias. Fifthly, each tumor tends to undergo a physiological cycle in that it has its growing, involuting and colloid or resting phases just as does the hyperplasia of physiologically adult tissue. This cycle is least evident in the fetal adenomas (which may persist throughout a long life with but little change) and progressively becomes more evident in the intermediate and in the simple adenomas.

In the absence of direct experimental proof of function, which is at present impossible, we believe that this evidence can scarcely be interpreted otherwise than that these tumor-like masses are only partially independent of the function of the gland as a whole.

## III.

## ANATOMICAL DESCRIPTION OF THE GROUP TYPES.

Group I. — The series of changes that take place in fully differentiated thyroid tissue as it passes from the normal through the many stages of active hyperplasia and on toward atrophy or involution have already been fully described in previous publications[11] and need not be reviewed here. Photomicrographs illustrating the normal thyroid (Figure 1), the hyperplastic phase (Figure 2), the involutionary phase (Figure 3), and the colloid or resting phase (Figure 4) are added for comparison with the corresponding phases of the tumor groups.

Group II. — These masses are most frequently seen in the so-called " nodular goiters " and make up a large proportion of " struma Knotchen," which occur commonly in the goiters of late childhood and puberty in endemic goiter districts. They are always multiple, rounded masses of varying sizes and in their growing phases always form integral parts of the general enlargement, but in their late stages may become more distinct and isolated through secondary metamorphoses. The true capsule is normally thin, often rudimentary, while in their late stages a false capsule of stretched atrophic non-tumor tissue may form a laminated covering several millimeters in thickness.

(*a*) (See Figure 5.) Histologically, the growing stage of a characteristic specimen is composed of follicles of variable size and often much larger than those of the non-tumor tissue. The lining epithelium is columnar and in the larger follicles has infoldings and plications similar to the non-tumor hyperplasia.. Between the larger follicles there are usually numerous smaller ones less completely differentiated. Colloid material, poorly staining and granular, is commonly present in all the larger follicles.

The stroma is less developed than in the non-tumor tissue and trabeculæ are absent. The blood supply in small tumors is in all respects similar to that in non-tumor tissue, but as the larger arteries run in the capsular tissues, growth, as Wölfler showed, occurs in the peripheral zone.

(*b.*) (See Figures 6a and 6b.) The central area of the mass is the oldest and shows the earliest involutionary changes. These consist of a decreased blood supply with hyaline changes in the scanty stroma; accumulation of colloid in the larger follicles and atrophic changes in the smaller follicles. The epithelium usually remains columnar.

(*c.*) (See Figure 7.) In the colloid phases, when uncomplicated, all the follicles appear relatively larger and more rounded, due to the return of the columnar epithelium to the cuboidal type and to the filling up of the follicular spaces with normally staining colloid. In tumors that have outgrown their blood supply the smaller follicles of the central area may be reduced in number and the larger ones acquire distortions in the hyalinized stroma so that it is difficult to distinguish colloid, and the epithelial cells may appear imbedded in a nearly uniform matrix. The blood vessels become thickened and much of the capillary structure of the interior may disappear.

Group III.— The intermediate tumors were grouped by Wölfler under fetal adenomas. We have arbitrarily introduced the group and under this name in order to emphasize the fact that between simple adenomas and the fetal adenomas there are many gradations.

These tumors are usually multiple and on section quite distinct in appearance from the non-tumor thyroid in that they are more cellular. They share the same variations in size and number that simple adenomas do, but have distinct well formed fibrous capsules.

(*a*). (See Figure 8.) In the growing phase the mass is grayish red in color. The parenchyma is soft, friable and composed of follicles closely set and more uniform in size than those of the simple adenomas. The lining epithelium may have slight infoldings and plications. The epithelium is high cuboidal or low columnar and usually regular in form. Colloid when present is granular and stains poorly. The stroma is less developed than in the simple adenomas. There are no trabeculæ. The largest vessels lie in the capsule and the larger vessels of the stroma have poorly developed coats. As growth proceeds hyaline degeneration and atrophy are more likely to take place in these than in the simple adenomas with their better organized blood supply.

(*b.*) (See Figure 9.) In the involutionary phase the follicles are more in evidence as colloid accumulates and the lining epithelium becomes more cuboidal.

As in the simple adenomas, the earliest involutionary changes take place in the central zone. Here there are larger colloid filled follicles surrounded by smaller atrophic follicles. The stroma is relatively increased and usually hyaline. In the sub-capsular zone there is also accumulation of colloid and in (*c*) (see Figures 10a and 10b) the colloid phase all the larger follicles are filled with uniformly staining colloid. The lining epithelium is low cuboidal and the larger vessels of the capsule are thickened. Atrophic or undeveloped follicles become more distinct as the stroma expands and in late stages may assume the appearance of a myxomatous adenoma (Wölfler).

Group IV. — Fetal adenomas are the best known and most distinct type. They are usually multiple and when their growth is arrested early may persist with but little change until old age. I have seen two autopsies in individuals over sixty years old where multiple adenomas from one to eight or ten millimeters in diameter have persisted with but little change (see Figures 11a, 11b, and 11c).

(*a.*) In gross appearance the growing stage of the minute tumors are indistinguishable from large lymph foci that may be present. The larger tumors are round, firm, and on section grayish opaque in color, usually in sharp contrast with the non-tumor thyroid. The tumor tissue is friable, very cellular, and there is no visible colloid. The capsule is well developed in all stages and there are no trabeculæ. Microscopically the stroma is very scant and undeveloped. The follicles are of uniform size, very small, closely set, rounded, and in most instances have visible lumina, though occasionally tumors are seen where this degree of differentiation has not occurred. The epithelial cells are small, cuboidal, uniform in size, and with hematoxylin the cytoplasm is usually tinged with blue. As a rule stainable colloid is absent. Wölfler[3] has shown that the blood supply is similar to that of normal fetal thyroid in richness and distribution about the follicles.

(*b.*) (See Figures 12a and 12b.) In the involutionary stage the follicles become more variable in size owing to different growth rates. The epithelium remains cuboidal and distinct lumina are present in all the more developed follicles. Colloid also appears in the follicular spaces. As with the other tumor groups differentiation is most advanced in the older central portion of the tumor mass and owing to the gradual reduction in blood supply the central areas show considerable variation in the number of follicles as a result of atrophy, hyaline degeneration and edema of the stroma, which in marked degrees correspond to Wölfler's myxomatous adenoma.

(*c.*) (See Figure 13.) The colloid phase is characterized by further accumulation of colloid in the follicles which may approach normal colloid in staining reactions. The follicular epithelium becomes low cuboidal. The blood supply is further reduced and secondary changes are most frequently seen in this group owing to their poorly developed blood supply.

To summarize the major features of the three tumor groups it is found that all have true capsules — often rudimentary in the simple adenomas, and progressively more prominent and distinct in the intermediate and fetal groups. The stroma is poorly developed and scanty in all — being least developed in the fetal adenomas. No trabeculæ are present, hence no lobulation as in the non-tumor tissue.

The larger well formed vessels lie in the capsules for the most part, while those of the parenchyma have poorly developed muscular and adventitial coats. As these tumors grow from the periphery their central portions are more and more removed from their blood supplies — an outgrowing of the blood supply, so to speak, which leads to atrophy, degeneration, and other more destructive metamorphoses. This tendency is most pronounced in the fetal adenomas and least so in the simple adenomas where the blood vessels are better developed.

The follicles are largest and most irregular in the simple adenomas and progressively become smaller and more regular toward the fetal adenomas. The epithelium in the growing phase is distinctly columnar in the simple adenomas and becomes more cuboidal in the intermediate and fetal adenoma groups. In the colloid phase all groups have cuboidal epithelium as the ruling type. Infoldings and plications are the rule in the simple adenomas, while they are rarely present in the intermediate and are absent from the fetal adenomas. The colloid content is much reduced during the growing phases of all groups and usually absent in the fetal adenomas, while in the colloid phases all developed follicles contain normally staining colloid.

## IV.

## SECONDARY AND TERMINAL METAMORPHOSES.

The frequency of complicating and destructive metamorphoses in these tumor-like masses has already been mentioned, but in order that one may not gain the impression from the earlier part of this paper, when we were considering as nearly uncomplicated examples as possible, that uncomplicated forms are the rule, we would repeat that as these tumors are seen at operation and at autopsy some secondary metamorphosis is usually present.

The same types of regressive changes are seen as well in the diffuse non-tumor thyroid overgrowths as in the strictly localized overgrowths. The frequency and extent of these changes progressively increase, however, as one passes from the diffuse non-tumor overgrowth through the simple, the intermediate, and the fetal adenomas. Just as destructive and degenerative changes are infrequently seen in the simple hyperplasias during their actively hyerplastic or growing stages, so with the tumor groups. It is during the regressive stages — involutionary and colloid — that these degenerative changes most frequently occur. Hence these changes are for the most part the result of old age, of decreased functional activity, and of failing nutrition in a tissue that normally is most active in early life. Mild degrees of these degenerative changes may normally be present in the senile gland. The frequency and extent to which they occur depend on the degree of thyroid overgrowth at the time when the increased physiological needs of the organism for thyroid activity are suddenly withdrawn. Thus mild degrees of thyroid hyperplasia, whether of tumor or non-tumor nature, may complete their physiological cycles without complications. This cycle for the non-tumor hyperplasia has been described elsewhere,[12] and for tumor it has been described under "Anatomical Descriptions of Tumors."

Of the strictly pathological metamorphoses occurring in tumors, hemorrhage (see Figure 16a) is the most common. As many observers, and particularly Wölfler, have shown, the capillaries of these tumors are, like normal thyroid capillaries, dilated and sinusoid with ampulla-like enlargements and thin walls. Through trauma, pressure, venous congestion, and failing nutrition these capillaries rupture ("Capillary Apoplexy" of Wölfler) and extravasations of blood take place, which when extensive may result in necrosis. Hemorrhages more commonly occur in the central portion of the tumor and the subcapsular zone usually is spared. If the hemorrhage is slight the stroma remains, from which healing by scar formation takes place and stellate scars of various sizes are of common occurrence in the centers of these tumors. Calcification often follows in these scars. When the destruction of tissue is extensive cyst formation results. The cysts may be single or multiple. Cysts of this origin are the surgical cysts of the thyroid as Bloodgood[13] and others have pointed out. Secondary hemorrhages into these cysts are relatively common even to the extent of producing large tumors.

Necrosis and cyst formation may also result from venous or arterial obstruction of the vessels as they pass through the capsule, either by thrombosis or by greatly increased capsular tension. In such cases there is acute, almost total necrosis associated clinically with sudden onset of pain and rapid enlargement in a few days. Examination of two such masses has shown the usual features of infarctions with liquefaction and extensive exudate into the encapsulated mass.

Edema is not uncommon and probably is dependent partly on peculiar locations of these tumors and, in consequence, the production of certain forms of circulatory stases — lymphatic and venous. If this edema is of long duration the stroma becomes myxomatous in type (see Figure 16b) and the gland acini often unduly separated. These are the myxomatous adenomas of Wölfler and others. Adenomas removed by enucleation are frequently edematous owing to venous stasis during operation, and this should not be confounded with spontaneous edema.

Atrophy (see Figure 15) of the alveoli is of frequent occurrence. There are all degrees of thinning out of the epithelial elements from slight rarefaction to total absence. This condition results from a slow impairment of nutrition through a relative or absolute decrease in the blood supply. It is always most marked at the center and gradually decreases toward the periphery, where often a zone of active, closely-set follicles is preserved. When the atrophy is complete, as occasionally occurs, one sees what some observers have called fibroma. The existence of true fibroma is acknowledged by some[14] and denied by others. I have seen three tumors that might be classed as fibroma, but considering all the facts they were without doubt fibromas through the persistence of the hardier tissue. There is little doubt that adenomas in certain locations cause a cessation of their growth by their own growth mechanically interfering with the blood supply and thus bring about fibrosis and atrophy.

The major cause of fibrosis and atrophy is probably the result of a systemic or physiological decrease in the blood supply which affects the gland as a whole.

Of the degenerations, hyaline (see Figures 14 and 15) is very common in the regressive phases of these tumors in association with atrophy of the glandular elements, with edema and with all the chronic impairments of nutrition. Like other evidence of failing nutrition affecting these tumors hyaline changes are usually more extensive in the center. As the blood vessels and gland alveoli disappear, the stroma assumes a firm, translucent, homogeneous appearance, which as Virchow pointed out may resemble cartilage grossly. Here and there blood spaces occur with only the endothelium intact and scattered extravasations of blood cells are seen. Alveoli, widely separated and distorted, are often present in the more hyaline areas and may appear as if the fatty epithelial cells were set directly in the hyaline matrix.

Amyloid infiltration is stated by many to be of common occurrence in these tumors. As it is not readily distinguishable from the extensive hyaline changes, there is some doubt as to its frequency.

Deposition of lime salts.—Carbonates, oxalates, and soaps are among the commonest of degenerative changes. There may be only the slightest traces of lime soaps in the hyaline stroma or the entire tumor nodule may be a single calcified mass. More commonly the carbonate deposits take place in the walls of large vessels in the capsule and of the capsule itself as in some cysts where the walls may be rigid and brittle from extreme lamellar calcification. The most common site for calcified areas is the central core either in the scar of a healed hemorrhage or in hyaline, fibrotic stroma secondary to atrophy of the gland follicles and may appear as single, pronged, solid or cavernous masses. More rarely in the early stages one may see a calcified meshwork outlining the stroma, in the meshes of which are well preserved gland follicles.

Infections. — Considering the favorable soil offered by these tumors in their many degrees and phases of failing nutrition, abscess formations are relatively rare, though they probably make up a majority of the acute thyroid abscesses. One of the older methods of treatment of these tumors was by the introduction of a seton which was followed by infection, suppuration and healing by scar formation. In influenza, scarlet fever, pneumonia, typhoid fever, tonsillitis, etc., these degenerating tumors are occasionally the seat of abscess formations.

Finally the development of malignant tumors, sarcoma, and carcinoma is in some way occasionally associated with these progressive and regressive tissue changes, especially of the adenomas. The so-called malignant adenoma is the most common type of thyroid carcinoma and is believed to originate in many cases from these tumors.

Summing up, then, it is found that the more common destructive metamorphoses include hemorrhage, necrosis, cyst formation; degenerations — amyloid, hyaline, calcareous, fatty; impairment of the blood supply and lymphatic drainage leading to necrosis in acute forms and to edema and atrophy in chronic stages, with myxomatous or simple fibrous changes secondary to atrophy of the glandular elements; and rarely infectious and malignant tumors.

These metamorphoses are common to both the tumor and the non-tumor tissue. They are seen for the most part in the functionally regressive stages. They are more common in the fetal adenomas and progressively decrease as one passes through the intermediate and simple adenomas to the simple hyperplasias.

## V.

## GENERAL DISCUSSION ON THE OCCURRENCE, DISTRIBUTION, AND ETIOLOGY OF THESE TUMORS.

These tumor-like masses occur with great frequency in man in all regions where endemic goiter occurs. In striking contrast one does not see them in the sheep, ox, pig, dog, cat, rabbit, or guinea-pig, although goiter is quite common in these animals in all goitrous regions. Bircher[15] has observed the development of "struma Knötchen" in rats during his "Kropfwasser" experiments. I have examined some of his preparations and there were many examples of circumscribed more or less encapsulated areas of hyperplasia closely resembling ordinary hyperplasia which could be classed morphologically as simple adenomas. The reaction with iodin had not been tried.

In the examination of large series of trout goiters one occasionally sees circumscribed areas of thyroid hyperplasia, but I have never seen them completely encapsulated as in man. To our knowledge these tumor-like masses have so far never been observed in monkeys, horses, goats, or any of the more accessible animals where considerable series of glands have been obtained. It is therefore singular that these tumor-like growths should be of such frequent occurrence in human goiter and so rare, if not absent, in the common domestic animals where the thyroid reactions are otherwise similar to those of man.

The age limits within which these tumors develop is of general etiological importance in that they develop only in early life, although they may be present (persistent) in the oldest individuals either as millet seed or pea-sized masses or reach considerable proportions. Tumors are occasionally seen well developed at birth as in cases reported by Wm. Müller.[16] Their development is believed to occur during all the years up to and including puberty. After puberty it is not probable that any new benign tumor anlagen arise and it is the belief of many that no new tumor anlagen arise after a much earlier period than puberty. The growth of existing tumor masses after puberty is subject to great variations just as simple hyperplasia. The factor of age is also related to the type of tumor. Thus the fetal type is the earliest to develop, although it may persist to old age. The simple adenomas are tumors of the more differentiated thyroid of childhood and possibly may develop up to and during the period of puberty. The age incidence of these tumors is also closely interwoven with the age incidence of hyperplasias in general. That is, both types of thyroid overgrowth occur most frequently between birth and puberty, and it is certain, as Virchow recognized, that these benign tumor-like masses do not occur in glands which are not also the seat of an universal hypertrophy or hyperplasia. The reverse, however, is not true, as one sees uniform hyperplasias at birth or at any time during life without the presence of tumors and in the hyperplasias after puberty as in pregnancy, exophthalmic goiter, menopause, etc., new tumors probably do not arise.

The incidence of sex is clinically that of goiter in general, hence the tumors under discussion are more common in the female. The sex differences are in large part due to the fact that the stimulus for tumor growth is the same or coincident with the stimulus for overgrowth of the gland as a whole and not that potential tumor anlagen are not equally common in both sexes in the developmental stages of the thyroid.

Of importance in this connection is the question whether tumor masses that have remained quiescent for a period can undergo a second period of growth just as the physiologically adult thyroid tissue commonly does. With what we know of their relations and the fact that they do undergo a modified cycle it is probable that they may undergo a second active hyperplasia in association with a second hyperplasia of the non-tumor thyroid, but as yet this has not been observed.

The question whether the stimulus to the growth of these tumors is identical with that which affects the whole gland can be answered positively with our present facts. The constant association of the generalized hyperplasia with their developmental stage, the age coincidence of both tumor and hyperplasia, the fact that they undergo a modified cycle of cell changes similar to that seen in the non-tumor tissue, the fact that these " Knotchen " develop in rats' thyroids as part of the general thyroid reaction, the fact that there are all gradations from the simple hyperplasias on the one hand to fetal adenomas on the other, the fact that the percentage iodin content relations and the effect of the administration of iodin are similar to those of the non-tumor thyroid overgrowth — all these facts favor the view that the stimulus to tumor growth is the stimulus that excites the thyroid as a whole to overgrowth.

Another question that may be discussed here is: What theory offers the simplest explanation of the mode of origin of this group of new growths? Billroth, Virchow, Wölfler, and all recent writers accept as the most probable explanation of the origin of fetal adenomas that they arise from embryonic cell rests. Groups of atrophic or undifferentiated cells lying in the stroma are familiar to all students of thyroid tissue and Wölfler was the first to point out their possible pathological significance. I think one can conclude on the evidence at hand that if fetal adenomas arise from these cell rests then all these circumscribed epithelial growths have a similar origin and the conception generally known as Cohnheim's hypothesis of tumor origin from cell rests as applied by Wölfler offers the clearest understanding of these particular growths. There are some modifications and restrictions to be mentioned. The thyroid has its embryomas (teratomas) and possibly tumors from " misplaced embryonic thyroid rests," but their modes of origin, as well as that of malignant tumors, are problems quite foreign to the one under discussion. These particular overgrowths are epithelial tumors which originate from true thyroid tissue and within the limits of the normal thyroid anlage. If one accepts the view that it is the same physiological stimulus which acts on both the tumor and non-tumor hyperplasia then it follows that one has to account only for the occurrence of cell rests of different degrees of anatomical differentiation from which these tumors arise. We must suppose that normally the size of the thyroid is determined by the physiological needs of the organism and as is well known this varies with the individual animal — its food, environment, geographical location, etc. The thyroid anlage in the course of its growth as with other body tissues is potentially capable of producing much more tissue than is usually needed, and in the course of its growth groups of cells from time to time become arrested in their growth just as is well known in the sex glands, muscles, etc. Normally these groups of cells are wholly absorbed in the course of time, but at any given period in the development of the thyroid there would be unabsorbed cell rests of different physiological ages and should a stimulus for increased functional activity become active during the period in the thyroid's life when these atrophic cell rests were still viable, then the whole gland would respond by both increased function and increased growth. The type of growth resulting would depend on the degree of physiological differentiation of the tissue from which the sudden and rapid growth began. Thus one could postulate that groups of cells were being arrested at all levels of physiological differentiation from the least in the fetal type to the highest in the fully differentiated adult type and a stimulus for increased activity would excite all these different physiological ages of thyroid cells to increased growth, which growth would take the form and function of the cell rest from which the growth started.

Such a view offers the most logical conception of the development of these growths and is the simplest conception that includes all the essential facts. It would presuppose that in the development of the thyroid, groups of cells were being continually marked for destruction either from the survival of the fittest, or through the physiological reduction of excess factors of safety, and there is evidence that something of this nature takes place. Thus the thyreoglossal tract normally is absorbed, but it frequently persists in goitrous districts with more or less thyroid tissue in its walls. So with the pyramidal lobe whose size and existence is directly related to the functional activity and overgrowth of the thyroid at an early period in life. In the dog the isthmus normally disappears, but in goitrous pups it usually persists.

Further, this theory would imply that these tumor-like masses functionate in proportion to the degree of differentiation and the evidence for this has already been stated.

This theory would imply that these growths took place only in early life if these cell rests normally undergo absorption as the thyroid differentiates. There is no evidence that thyroid overgrowths developing from normal glands after puberty as, for example, the overgrowths of pregnancy, menopause, exophthalmic goiter, infectious diseases, ever develop adenomas. The theory would also imply that there should be all gradations histologically from the lowest or fetal type to the highest or fully differentiated overgrowth. There are such gradations.

This conception would explain the usual occurrence of multiple tumors and often the occurrence of tumors of different physiological ages in the same gland. It would also imply that fetal adenomas are less commonly observed and simple adenomas are more commonly observed as age advances up to the age when no new tumor formations develop. There is no experimental proof as yet, but the clinical evidence tends to support these age variations.

Of objections to the modification of Cohnheim's hypothesis applied to these particular thyroid growths the strongest to my mind is the fact that similar tumors are not found in the ordinary domestic animals, and on the basis of such an hypothesis how do they escape, since their thyroids in other reactions are so similar to those of man? The same question would arise also in the case of fibromyomata of the uterus — why they should be so uncommon in the lower animals whose uteri have many anatomical and physiological attributes in common with the human uterus?

Finally the question arises, are these benign, encapsulated, epithelial growths to be considered as tumors? The physiological overgrowth of fully differentiated thyroid tissue is in no sense tumor, while the fetal adenoma lacks many of the attributes associated with the fully differentiated tissue and there is a continuous gradation between these two extremes.

If the fetal adenoma is a tumor, as most observers agree, then we must recognize different degrees of tumor, — "partial tumors," so to speak, depending on the degree of physiological independence of the growth. On such a basis all these growths are only partial tumors since all have attributes in common with each other and in common with non-tumor thyroid overgrowths.

Of the two means at present of recognizing these tumors, (1) morphology and (2) the reaction with iodin, we would lay more stress on the more delicate and constant iodin reaction and would tentatively group as tumors all encapsulated epithelial growths which did not react with iodin in the time limits in which the non-tumor tissue reacted. We therefore recognize these growths as benign or "partial tumors," in the sense that they are functionally only partially independent structures.

## VI.

### TABULATION NO. 4. ALL CASES.

*Iodin per gram of dried gland in milligrams.*

| | | Non-tumor Thyroid. | Adenoma. |
|---|---|---|---|
| I. Fetal adenomas. | | | |
| (1) Growing phase: extremes | | 2.78 | 0.31 |
| | | 0.17 | 0.00 |
| 20 cases | mean | 0.65 | 0.07 |
| | average | 1.02 | 0.09 |
| (2) Involutionary phase: extremes | | 2.18 | 0.69 |
| | | 0.21 | 0.05 |
| 15 cases | mean | 0.82 | 0.18 |
| | average | 0.89 | 0.25 |
| (3) Colloid phase: extremes | | 1.62 | 1.31 |
| | | 0.14 | 0.32 |
| 5 cases | mean | 1.31 | 1.07 |
| | average | 1.02 | 0.94 |
| II. Intermediate adenomas. | | | |
| (1) Growing phase: extremes | | 1.28 | 0.37 |
| | | 0.75 | 0.08 |
| 8 cases | mean | .......... | 0.22 |
| | average | 1.01 | 0.23 |
| (2) Involutionary phase: extremes | | 4.67 | 0.81 |
| | | 0.38 | 0.15 |
| 11 cases | mean | 2.14 | 0.41 |
| | average | 1.93 | 0.46 |
| (3) Colloid phase: extremes | | 1.93 | 1.03 |
| | | 0.50 | 0.09 |
| 8 cases | mean | 0.74 | 0.36 |
| | average | 0.98 | 0.45 |

TABULATION NO. 4. — *Continued.*

| | | Non-tumor Thyroid. | Adenoma. |
|---|---|---|---|
| III. Simple adenomas. | | | |
| (1) Growing phase: extremes | | 2.14 | 1.63 |
| | | 0.43 | 0.06 |
| 6 cases | mean | 1.57 | 0 28 |
| | average | 1.31 | 0.59 |
| (2) Involutionary phase: extremes | | .......... | 0.54 |
| | | .......... | 0.20 |
| 3 cases | mean | .......... | 0.31 |
| | average | 0.77 | 0.35 |
| (3) Colloid phase: extremes | | 2.31 | 2.50 |
| | | 0.06 | 0.18 |
| 17 cases | mean | 1.00 | 0.52 |
| | average | 1.08 | 0.65 |

TABULATION No. 5. CASES NOT FED IODIN.

*Iodin per gram of dried gland in milligrams.*

| | Non-tumor Thyroid. | Adenoma. |
|---|---|---|
| I. Simple adenomas. | | |
| (1) Growing phase: extremes | 0.43 | 0.29 |
| | 0.43 | 0.06 |
| 2 cases mean | .......... | ...... |
| average | 0.43 | 0.18 |
| (2) Involutionary phase: extremes | .......... | 0.54 |
| | .......... | 0.20 |
| 3 cases mean | .......... | 0.31 |
| average | 0.77 | 0.35 |
| (3) Colloid phase: extremes | 1.34 | 0.77 |
| | 0.06 | 0.18 |
| 14 cases mean | 0.62 | 0.48 |
| average | 0.69 | 0.46 |
| II. Intermediate adenomas. | | |
| (1) Growing phase: extremes | .......... | 0.37 |
| | .......... | 0.08 |
| 7 cases mean | .......... | 0.20 |
| average | 0.75 | 0.22 |
| (2) Involutionary phase: extremes | 0.54 | 0.79 |
| | 0.38 | 0.23 |
| 7 cases mean | 0.41 | 0.41 |
| average | 0.44 | 0.45 |
| (3) Colloid phase: extremes | 1.15 | 0.86 |
| | 0.50 | 0.09 |
| 7 cases mean | 0.66 | 0.35 |
| average | 0.74 | 0.36 |

Tabulation No. 5.—*Continued.*

| | Non-tumor Thyroid. | Adenoma. |
|---|---|---|
| III. Fetal adenomas. | | |
| (1) Growing phase: extremes | 0.96 | 0.31 |
| | 0.17 | 0.00 |
| 15 cases: mean | 0.34 | 0.09 |
| 15 cases: average | 0.46 | 0.11 |
| (2) Involutionary phase: extremes | 1.11 | 0.69 |
| | 0 21 | 0.05 |
| 13 cases: mean | 0.57 | 0.16 |
| 13 cases: average | 0.60 | 0.25 |
| (3) Colloid phase: extremes | ......... | 1.25 |
| | .......... | 0.32 |
| 2 cases: mean | .......... | ....... |
| 2 cases: average | 0.14 | 0.78 |

### TABULATION NO. 6. CASES FED IODIN.

*Iodin per gram of dried gland in milligrams.*

| | Non-tumor Thyroid. | Adenoma. |
|---|---|---|
| I. Simple adenomas. | | |
| (1) Growing phase: extremes | 2.14 | 1.63 |
| | 1.54 | 0.27 |
| 4 cases, mean | 1.65 | 0.65 |
| 4 cases, average | 1.75 | 0.80 |
| (2) Involutionary phase: | | |
| 0 cases. | | |
| (3) Colloid phase: extremes | 2.31 | 2.50 |
| | 1.58 | 1.85 |
| 3 cases, mean | | 2.31 |
| 3 cases, average | 1.95 | 2.22 |
| II. Intermediate adenomas. | | |
| (1) Growing phase: | | |
| 1 case — average | 1.28 | 0.32 |
| (2) Involutionary phase: extremes | 4.67 | 0.81 |
| | 2.14 | 0.15 |
| 4 cases, mean | 2.69 | 0.47 |
| 4 cases, average | 3.05 | 0.47 |
| (3) Colloid phase. | | |
| 1 case — average | 1.93 | 1.03 |

TABULATION NO. 6. — *Continued.*

| | | Non-tumor Thyroid. | Adenoma. |
|---|---|---|---|
| III. Fetal adenomas. | | | |
| (1) Growing phase: extremes | | 2.78 | 0.14 |
| | | 1.19 | 0.00 |
| 5 cases | mean | 1.46 | 0.07 |
| | average | 2.02 | 0.06 |
| (2) Involutionary phase: extremes | | 2.18 | 0.31 |
| | | 1.34 | 0.20 |
| 2 cases | mean | .......... | ....... |
| | average | 1.76 | 0.26 |
| (3) Colloid phase: extremes | | 1.62 | 1.31 |
| | | 1.31 | 0.77 |
| 3 cases | mean | .......... | 1.07 |
| | average | 1.47 | 1.05 |

TABLE 2

| NUMBER OF EXPERIMENT | CONTROL OF JOINTS | RESPONSE OF LEG TO POINT | RESPONSE OF PAW TO HEAT (PAIN) | REFLEXES | | SPHINCTERS | | CORD AND ROOT INVOLVEMENT (See diagrams) | POSITIVE CONCLUSIONS FOR INDIVIDUAL |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Knee jerk | Anal | Anus | Bladder | | |
| 64 | Paralysis of dorsal flex. of left paw and ankle<br>Direct stimulation evoked no response in left E. P. | Poor on both legs | No test | Absent on left | Sluggish | N | N | In upper 7th L. seg. post. horns and cols. on both sides.<br>On left, lat. col. and upper $\frac{2}{3}$ of ant. horn. In 1st S. seg. cent. part of grey matter on left. In 5th, 6th, 7th and 1st S. seg. postero-mesial col. 5th and 6th post. roots. | Dorsal flexion of paw and ankle (E. P.), in dorsal part of ant. horn of upper 7th L. |
| 78 | Paralysis of dorsal flexion of right paw and ankle. Weak extension of rt. hip and knee<br>Direct stimulation evoked no response in right E. P. | Poor on rt. leg | Poor on right | Absent on right | Sluggish | N | N | In mid. 4th L. seg., lower part of rt. ant. horn and adjoining col. In high 5th L. seg., entire rt. ant. horn and adjoining col. In low. 5th L. seg., entire rt. half of cord except ant. mesial col. In mid. 6th L. seg., whole cord except ant. cols. and lower part of lt. lat. In upper 7th rt. ventromesial col. very narrow 4th, 5th and 6th rt. ant. roots. | Dorsal flexion of paw and ankle (E. P.)—in lower 6th L.<br>I. P. control lower (in 7th)<br>Extens. of hip and knee in 5th<br>Gap between centers of lower and upper leg, perhaps containing centers of hamstrings |
| 86 | Paralysis of rt. paw and ankle and of dorsal flex. of left paw and ankle. Weak extension of rt. hip and knee and some weakness of lt. hip and knee<br>Direct stimulation evoked no response in either E. P. and very slight (of toes only) in rt. I. P. | Poor on rt. leg | Poor on right | Absent on right<br>Weak on left | Sluggish | N | Weak | In upper 5th L. seg., rt. side of cord except pyram. tract, also lt. ant. horn. In low. 5th L. seg. rt. side of cord. In mid. 6th whole cord except lt. ant. col. and small part of lt. ant. horn. In upper 7th inner $\frac{2}{3}$ of rt. ant. horn.<br>5th and part of 6th rt. and lt. ant. and post. roots and 4th rt. ant. root. | Dorsal flex. of paw and ankle (E. P.) in mid. 6th<br>Plantar flex. of paw and ankle (I. P. ) in inner dorsal part of ant. horn of upper 7th<br>Extens. of hip and knee in 5th |
| 69 | Slight weakness in dorsal flex. of rt. paw and ankle | N | N | N | Sluggish | N | N | In mid. 6th all of grey matter and dorsal and ventral cols. which are partly spared on left. In upper 7th rt. ant. horn, rt. lat. col., both ant. cols. and lower $\frac{2}{3}$ of lt. ant. horn. 6th rt. post. and 6th and 7th rt. ant. roots. | Dorsal flex. of paw and ankle (E. P. ) partly in upper 7th.<br>Gap between centers of lower and upper leg |
| 65 | Paralysis of entire left leg except for flex. of hip. Weak extension of knee and weak flex. of hip on right | Poor on left | No test | Absent on left<br>Weak on right | Sluggish | N | N | In upper 6th whole left of cord, and dorso- and ventro-mesial cols. and inner part of grey matter on rt. In low. 6th lt. ant. horn and lt. antero-lat. col.; also inner part of rt. ant. horn. In low. 7th outer part of lt. ant. horn and lt. antero-lat. col. In 2d Sac. grey matter about central canal. 5th 6th, 7th and 1st lt. ant. roots; 5th and 6th lt. post. roots; 6th rt. ant. root. | Control of most of leg in 5th seg. and those below<br>Point sensation passes through 5th post. root |
| 75 | Weakness of gluteals and hamstrings on rt. hip flexed and knee straight and stiff<br>Paw paralyzed and flaccid | Poor on right | Poor on right | Absent on right | N | N | N | In mid. 6th dorsal cols. and inner part of base of rt. post. horn. In upper 7th rt. side of cord and dorsal col. and inner part of grey matter on lt. In 1st Sac. rt. side of cord and inner part of lt. ant. horn. 6th and 7th rt. post. horns. | Sciatic centers in 7th L. and 1st Sac. |
| 70 | Spastic loss of control of hind parts from pelvis down. Weakness of erector spinae, glutei and quadriceps. Hamstrings fairly good | Poor on left | N | N | N | N | N | In mid. 4th rt. ant. horn. In upper 5th lt. post. and all of antero-mesial cols. rt. ant. horn, most of lt. grey matter. In mid. 5th all of cord except rt. post. horn. In low. 5th whole cord. In high 6th lt. of cord. In mid. 6th ant. cols. and lower part of ant. horns. 4th, 5th, and part of 6th rt. and lt. ant. roots 4th lt. post. root. | Some crural and gluteal centers in upper 5th. Hamstrings below mid. 6th<br>Transection in lower 5th cuts off brain control of lower leg<br>Point sensation passes through 4th post. root<br>Heat-pain sensation above mid. 5th<br>K.J. anal reflex, control of sphincters below upper 6th |
| 72 | Flaccid paralysis of hind parts from pelvis down. | Poor on both | No test | Absent on both | No test | Weak | Weak | In mid. 5th, base of dorsal cols. part of rt. ant. root. In low. 5th, all of grey matter and dorsal and vent. cols. except left post. horn and small part of vent. col. adjoining. In mid. 6th all of grey matter and rt. ventral and lat. cols. In mid. 7th rt. ant. horn and inner part of lt. grey matter. 5th, 6th and part of 7th rt. lt. ant. roots, rt. 5th post. root. | Control of hind parts including sensation, K.J.'s and sphincters in 5th to 7th inclusive |
| 68 | Paralysis of tail and slight weakness in dorsal flex. of rt. paw. | Poor on tail<br>On legs N | Poor on tail | N | Sluggish | Weak | Weak | In mid. 6th dorso-mesial col. In mid. 7th rt. side of cord, dorso- and ventro-mesial cols. and inner part of lt. grey matter. In low 1st Sac., whole of cord (conus). 5th and 6th rt. ant. and post. roots. | Control of motion and sensation to point and heat-pain in tail in 7th L. and 1st S.<br>Anal reflex and control of sphincters in 7th L. and 1st Sac. |
| 73 | Paralysis of tail | Poor on tail | Poor on tail | N | Sluggish | N | Weak | In upper 7th, all of grey matter except lt. post. horn; also vent. cols. and lower part of rt. lat. col. and rt. dorsal col. In lower 7th entire cord (conus). | Control of motion and sensation to point and heat-pain in tail in 7th L.<br>Anal reflex and control of bladder in 7th L. |

Letter N = normal. E. P. = external popliteal. I. P. = internal popliteal

## TABLE No. 8.

| Series No. Pathological No. Sex, Age. | Autopsy or Surgical, Hospital. | Intermediate Adenomas. |  |  |  | Anatomical State of the Non-tumor Tissue. | Iodin Contents. In milligrams per gram dried. |  | Remarks. |
|---|---|---|---|---|---|---|---|---|---|
|  |  | Growing Phase. | Involutionary Phase. | Colloid Phase. | Complication. |  | Non-tumor Thyroid. | Adenoma. |  |
| H. 291.<br>4090 G.S.<br>Female, 30. | Surgical.<br>Lakeside. | + |  |  | None. | — | — | 0.23 | Enucleation of adenoma from right lobe on account of deformity. |
| H. 87.<br>Female. | Surgical.<br>Charity. | + |  |  | Beginning cyst formation, hemorrhage, calcification. | — | — | — |  |
| H. 142.<br>—<br>Male, 65. | Autopsy<br>No. 802.<br>Lakeside. | + |  |  | None. | Moderate glandular hyperplasia. | — | — | Dead of peritonitis secondary to operation for artificial anus. Multiple adenomas in thyroid, general thyroid enlargement. |
| H. 442.<br>2552 P.W.S.<br>Female. | Surgical.<br>Lakeside. | + |  |  | Practically a cyst with thin zone of adenoid tissue, subcapsular. | — | — | 0.08 | Tumor, enucleated, 8 x 8 cm. |
| H. 459.<br>2778 P.W.S.<br>Male, 41. | Surgical.<br>Lakeside. | + |  |  | None. | — | — | 0.37 | Old adenoma in generally enlarged thyroid. |
| H. 544.<br>8620.<br>Female. | Surgical.<br>Lakeside. | + |  |  | None. | Colloid. | 1.28 | 0.32 | Clinically exophthalmic goiter. Ligated superior thyroid arteries and later removed lobe. Given iodin. |
| H. 549.<br>8668.<br>Female. | Surgical.<br>Lakeside. | + |  |  | Cystic center. | — | — | 0.20 | Enucleated, orange-sized adenoma. |
| H. 294.<br>—<br>Female, 33. | Autopsy<br>No. 981.<br>Lakeside. |  | + |  | None. | Colloid. | 2.92 | 0.15 | General peritonitis secondary to ectopic gestation. Two cherry-sized adenomas. General thyroid enlargement. |
| H. 329.<br>1797 P.W.S.<br>Female, 34. | Surgical.<br>Lakeside. |  | + |  | None. | — | — | 0.79 | Two adenomas, enucleated, roughly 3 x 3-4.5 x 4 cm. |
| H. 460.<br>2807 P.W.S.<br>Female. | Surgical.<br>Lakeside. |  | + |  | Hyaline degeneration of stroma, edemas. | Colloid, early, glandular hyperplasia. | 0.54 | 0.31 | Much enlarged lobe containing an adenoma 5.5 x 6 cm. |
| H. 466.<br>2870.<br>Female. | Surgical.<br>Lakeside. |  | + |  | Dense hyaline scar-like core. | — | — | 0.38 | Thick capsuled adenoma 7 x 7 cm. Enucleated. |
| H. 480.<br>8043.<br>Female. | Surgical.<br>Lakeside. |  | + |  | Cystic, zone of adenoid tissue, 1–2 cm. | Colloid. | 4.67 | 0.81 | Fed iodin for long time. Moderately enlarged lobe removed. |
| H. 546.<br>8662. | Surgical.<br>Lakeside. |  | + |  | None. | — | — | 0.54 | Uniform involuting adenoma, enucleated. |
| H. 566.<br>Female, 34. | Surgical.<br>Charity. |  | + |  | Slight hemorrhage, early cystic change. | — | — | 0.49 | Three small adenomas, enucleated. |
| H. 543.<br>8619.<br>Female. | Surgical.<br>Lakeside. |  | + |  | Hemorrhagic, cystic. | Atrophic colloid, early. | 0.41 | 0.23 | One lobe removed contains one large adenoma, cystic, and several smaller ones. |
| H. 565.<br>8746.<br>Female. | Surgical.<br>Lakeside. |  | + |  | Cystic, hemorrhage, calcification. | Colloid. | 2.14 | 0.23 | One lobe removed, slightly enlarged. Fed iodin. |
| H. 372.<br>1945 P.W.S.<br>Female. | Surgical.<br>Lakeside. |  |  | + | Hyaline degeneration, multiple cystic areas. | Colloid. | 1.15 | 0.25 | One lobe removed containing adenoma 6 x 6 cm. Much lymphoid tissue throughout stroma of gland. |
| H. 340.<br>—<br>Male, 55. | Autopsy<br>No. 1007.<br>Lakeside. |  |  | + | Calcified fibrous core in each adenoma. | Colloid. | 1.93 | 1.03 | Patient dead of arteriosclerosis and myocarditis. Symmetrical colloid goiter and two adenomas. Given potassium iodid for some time. |
| H. 436.<br>2486 P.W.S.<br>Female. | Surgical.<br>Lakeside. |  |  | + | Hemorrhagic, cystic, calcareous changes. | Colloid. | — | 0.35 | Highly complicated. Multiple adenomatous goiter. |
| H. 430.<br>2445 P.W.S.<br>Female. | Surgical.<br>Lakeside. |  |  | + | Extensive cysts, scars and degenerations. | Colloid, early glandular hyperplasia. | 0.74 | 0.09 | Entire lobe much enlarged. Exceedingly complex pathological changes, atrophy degeneration, tumors, etc. |
| H. 492.<br>8778.<br>Female, 48. | Surgical.<br>Lakeside. |  |  | + | Calcification of capsule, several hemorrhages. | — | — | 0.86 | Three adenomas, enucleated. Iodin for six months under my direction. Duration of adenomas over forty years. |
| H. 563.<br>8748. | Surgical.<br>Lakeside. |  |  | + | Calcified, fibrous core. | — | — | 0.38 | Two calcified adenomas, enucleated. |
| H. 575.<br>8826.<br>Female. | Surgical.<br>Lakeside. |  |  | + | Very large follicles, slight hemorrhage. | Atrophic colloid, early glandular hyperplasia. | 0.57 | 0.46 | Old complicated adenomatous goiter. |
| H. 590.<br>8897.<br>Female. | Surgical.<br>Lakeside. |  |  | + | Slight hemorrhage. | Atrophic colloid. | 0.50 | 0.18 | Old multiple adenomas in generally enlarged thyroid. |
| H. 607.<br>9018.<br>Female. | Surgical.<br>Lakeside. |  | + |  | Colloid visible, some calcification. | Atrophic (tension) colloid. | 0.38 | 0.41 | Old long-standing goiter. Lobe contains five adenomas in different stages of involution. |
| H. 612.<br>9042.<br>Female. | Surgical.<br>Lakeside. | + |  |  | Very slight extravasation of blood (recent). | Atrophic colloid. | 0.75 | 0.20 | Adenoma 4 x 5 cm. Soft gray-red friable tissue. Only one in the tissue removed. |
| H. 611.<br>9041.<br>Male. | Surgical.<br>Lakeside. |  | + |  | None. | Pure colloid. | 2.46 | 0.71 | Lobe enlarged. Nodule contains two adenomas, (1) 1.5 x 1.5 and (2) 3 x 3 cm. |

### Table No. 9.

| Series No. Pathological No. Sex, Age. | Autopsy or Surgical Hospital. | Fetal Adenomas. | | | | Anatomical State of the Non tumor Thyroid Tissue. | Iodin Contents. In milligrams per gram dried. | | Remarks. |
|---|---|---|---|---|---|---|---|---|---|
| | | Growing Phase. | Involutionary Phase. | Colloid Phase. | Complications. | | Non tumor Thyroid. | Adenoma. | |
| H. 3.<br>—<br>Female, 50. | Autopsy No. 651. Lakeside. | + | | | None. | Colloid. | — | — | Gland symmetrically enlarged, multiple small adenomas. |
| H. 11.<br>859 P.W.S. | Surgical. Lakeside. | + | | | Cystic center. | Colloid. | — | — | Tumor 6 x 4 cm., cyst wall 1 mm. Zone of glandular tissue beneath capsule 0.5–1.0 cm. thick. |
| H. 66.<br>—<br>Female, 42. | Autopsy No. 658. Lakeside. | + | | | Early diffuse calcification of tumor stroma. | Moderate, glandular hyperplasia. | — | — | Multiple small adenomas in a slightly enlarged thyroid. Patient dead of peritonitis. |
| H. 239.<br>P.W.S.<br>Female, 35. | Surgical. Lakeside. | + | | | None. | Colloid. | 1.19 | 0.14 | Clinically a "mild Graves," tumor size of hen's egg in right lobe, parts of both lobes removed. |
| H. 240.<br>Male, 45. | Autopsy No. 934. Lakeside. | + | | | None. | Colloid, senile, atrophy, sclerosis. | 1.46 | 0.01 | Small atrophic thyroid with five pea-sized adenomas. |
| H. 242.<br>Male, 68. | Autopsy. City Hospital. | + | | | Calcification of thyroid arteries, extensive. | Colloid, senile. | 2.78 | 0.07 | One egg-sized adenoma, several large "colloid cysts." |
| H. 348.<br>Female. | Surgical. Lakeside. | + | | | None. | Normal, colloid. | 1.92 | 0.00 | Entire lobe removed, tumor size of golf ball. |
| H. 359.<br>1893 P.W.S.<br>Female. | Surgical. Lakeside. | + | | | None. | Colloid. | 2.77 | 0.08 | Slight general thyroid enlargement. Tumor 3 x 3 cm. Fed iodin. |
| H. 370.<br>1928 P.W.S.<br>Female. | Surgical. Lakeside. | + | | | Cystic center, hemorrhagic, edematous. | — | — | 0.07 | Cystic adenoma 7 x 7 cm., enucleated. |
| H. 434.<br>—<br>Female. | Surgical. Lakeside. | + | | | None. | Normal, colloid. | 0.96 | 0.03 | One lobe, weight 10 grams, containing adenoma 2 x 2 cm. Clinically — "Exophthalmic goiter." |
| H. 438.<br>2504 P.W.S.<br>Male. | Surgical. Lakeside. | + | | | Cystic center, hyaline necrosis, calcification. | Colloid, early, moderate glandular hyperplasia. | 0.34 | 0.14 | Entire lobe removed. Cystic adenoma 5 x 5 cm. |
| H. 440.<br>4751 G.S.<br>Female. | Surgical. Lakeside. | + | | | None. | — | — | 0.00 | Enucleated. |
| H. 446.<br>2682 P.W.S.<br>Female. | Surgical. Lakeside. | + | | | None. | Tension atrophy, colloid. | 0.31 | 0.00 | Right lobe removed. Adenoma 6 x 6 cm. imbedded in lobe. |
| H. 454.<br>4913 G.S.<br>Female. | Surgical. Lakeside. | + | | | None. | Colloid, early, glandular hyperplasia. | 0.53 | 0.11 | Fed desiccated thyroid. Adenoma 4 x 4 cm. imbedded. |
| H. 457.<br>2733 P.W.S.<br>Male, 69. | Surgical. Lakeside. | + | | | Acute hemorrhage into adenoma. | Colloid, early, moderate glandular hyperplasia. | 0.31 | 0.01 | Right lobe removed. Recent hemorrhage into adenoma already cystic. |
| H. 461.<br>2809 P.W.S.<br>Female. | Surgical. Lakeside. | + | | | None. | Colloid. | 0.77 | 0.18 | One lobe removed, very slightly enlarged. Two fetal adenomas and much lymphoid tissue in stroma. |
| H. 482.<br>8047.<br>Female. | Surgical. Lakeside. | + | | | Hemorrhage, hyaline necrosis, calcification. | Colloid, early, glandular hyperplasia. | 0.38 | 0.18 | Adenoma size of goose egg with arborescent calcification of stroma. |
| H. 506.<br>8285.<br>Female. | Surgical. Lakeside. | + | | | Almost completely cystic. | — | — | 0.04 | Enucleated large cystic adenoma and thin subcapsular zone of adenoma tissue. |
| H. 534.<br>8542.<br>Female. | Surgical. Lakeside. | + | | | Cystic center, hyaline necrosis, hemorrhage. | Tension atrophy. | 0.34 | 0.24 | Large hemorrhagic cystic adenoma in an otherwise slightly adenomatous gland. |
| H. 536.<br>8568<br>Male. | Surgical. Lakeside. | + | | | Old fibrous core. | Almost complete tension atrophy. | 0.17 | 0.31 | Large hard fibroid adenoma. Non-tumor thyroid, almost wholly fibrous tissue from pressure atrophy. |
| H. 141.<br>3434 G.S.<br>Female, 11 yrs. | Surgical. Lakeside. | | + | | None. | Early, moderate glandular hyperplasia. | — | — | Right lobe removed. Adenoma 5 x 5 cm. Whole thyroid enlarged. |
| H. 217.<br>1450 P.W.S.<br>Female, 41. | Surgical. Lakeside. | | + | | None. | Colloid. | 1.34 | 0.20 | Whole lobe slightly enlarged, removed. Capsule thick, stroma of adenoma is hyaline, thickened and possibly edematous. |
| H. 336.<br>1825 P.W.S.<br>Female, 30. | Surgical. Lakeside. | | + | | Hemorrhage, cystic center. | Colloid, early, glandular hyperplasia. | 0.82 | 0.17 | Old highly complicated goiter and one cystic adenoma. |
| H. 377. | Surgical. German Hospital. | | + | | Large, firm, fibrous, scarlike center. | — | — | 0.05 | Encapsulated old adenoma 7 x 7 cm., enucleated. |
| H. 380<br>4401 G.S.<br>Male, 35. | Surgical. Lakeside. | | + | | Edematous, fibrous center. | Colloid. | — | 0.07 | Enucleation of tumor with a small bit of original thyroid tissue for histology. |
| H. 423.<br>2360 P.W.S.<br>Female. | Surgical. Lakeside. | | + | | Cyst wall and subcapsular zone of adenoma. | — | | 0.15 | Enucleation of cystic adenoma containing much changed blood and cholesterin. |
| H. 465.<br>2845 P.W.S.<br>Female. | Surgical. Lakeside. | | + | | Hyaline degeneration, calcified central scar, edema. | Colloid, early, glandular hyperplasia, tension atrophy. | 0.82 | 0.31 | Highly complicated old adenoma. |
| H. 484.<br>8084 G.S.<br>Female. | Surgical. Lakeside. | | + | | None. | Colloid. | 2.18 | 0.31 | Patient was given iodin for 3 weeks before operation. |
| H. 514<br>8356.<br>Female. | Surgical. Lakeside. | | + | | Hyaline degeneration, cystic areas. | — | — | 0.31 | Enucleated, 5 x 5 cm. Some colloid visible. |
| H. 528.<br>—<br>Female. | Surgical. German Hospital. | | + | | Highly hemorrhagic, hyaline necrosis, fibrotic core. | Colloid, marked tension atrophy. | 0.32 | 0.15 | Entire right lobe containing adenoma 8 x 8 cm. Original thyroid stretched and atrophic about this mass. |
| H. 567.<br>—<br>Female. | Charity Hospital. Surgical. | | + | | Hemorrhagic, edematous. | — | — | 0.46 | Three adenomas enucleated. |
| H. 570.<br>8774.<br>Female. | Surgical. Lakeside. | | + | | Slight fibrosis. | Stretched atrophic colloid. | 0.31 | 0.11 | Very large, long-standing goiter and several adenomas in various phases. |
| H. 599.<br>8958.<br>Female. | Surgical. Lakeside. | | + | | General hyaline necrosis and fibrosis, calcification. | Colloid. | 1.11 | 0.15 | Right lobe removed. Slight general enlargement. Adenoma 4 x 4 cm. |
| H. 600.<br>Female, 40. | Surgical. Charity. | | + | | Slight hemorrhage and cyst formation. | — | | 0.34 | Two adenomas were enucleated from a generally enlarged thyroid; also excellent thyreoglossal tract from hyoid bone to thyroid isthmus. |
| H. 453.<br>4900 G.S.<br>Female. | Surgical. Lakeside. | | | + | None. | — | | 1.07 | Fed iodin for 18 months. Several adenomas present in gland. |
| H. 488.<br>8178.<br>Female, 48. | Surgical. Lakeside. | | | + | Cystic center. | Atrophic colloid. | 1.62 | 0.77 | One lobe removed. Moderately enlarged adenoma 6 x 6 cm. |
| H. 558.<br>8729.<br>Female. | Surgical. Lakeside. | | | + | None. | — | — | 1.25 | Adenoma 4 x 4 cm., enucleated. No complication. |
| H. 521.<br>8414.<br>Female. | Surgical. Lakeside. | | | + | Fibrous core, hemorrhage. | Very atrophic, tension atrophy. | 0.14 | 0.32 | Adenoma size of indoor baseball surrounding thyroid, highly atrophic. |
| H. 568.<br>8780.<br>Female. | Surgical. Lakeside. | | | + | Multiple small adenomas. | Colloid. | 1.31 | 1.31 | Fed iodin off and on for two years. Slight general thyroid enlargement. |
| H. 613.<br>9034.<br>Male. | Surgical. Lakeside. | | + | | Cystic, calcified. | Colloid, early, moderate glandular hyperplasia. | 0.21 | 0.69 | Adenoma 6 x 6 cm. single, cystic, with zone of adenoma tissue 1.5 cm. thick beneath capsule, calcified. |

## VII.

## SUMMARY.

There are all gradations between strictly non-tumor, simple parenchymatous overgrowths at one end and true fetal adenomas at the other end of the series.

By comparing the percentage iodin contents of these tumors with their structure, a general relationship can be made out which is similar to that noted in the non-tumor overgrowths in that they have growing, involutionary, and colloid or resting phases.

Neither the structure of the tumor nor its iodin content bear any essential relationship to the non-tumor tissue of the same gland save that these tumors are not seen apart from a general hypertrophy or hyperplasia and therefore are not strictly independent growths. The most marked evidence of independence is seen in the fetal adenomas and progressively lessens toward the non-tumor hyperplasias.

Cohnheim's conception offers the best explanation of the origin of these tumors when one enlarges it to include the conception (1) that there are potential tumor anlagen formed at different physiological ages of the development of the main thyroid mass and (2) that the stimulus for tumor growth is the same as for that of the thyroid as a whole. These growths may tentatively be considered as "partial tumors."

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## EXPLANATION OF PLATES.

PLATE XV., FIG. 1. — Normal adult thyroid. x 90.

FIG. 2. — Active hyperplasia from physiologically adult thyroid tissue. x 90.

FIG. 3. — Involution of active hyperplasia. Note relative enlargement of follicles; accumulation of colloid. x 90.

FIG. 4. — Colloid or resting state — complete involution. Note cuboidal epithelium, uniform colloid. x 90.

FIG. 5. — Growing or actively hyperplastic stage of simple adenoma. x 75.

(*a*) Capsule.
(*b*) Parenchyma of tumor.

FIG. 6. — Involutionary stage of simple adenoma. Note thin vacuolated colloid and enlarged irregular follicles with high cuboidal epithelium. x 90.

(*a*) Capsule.
(*b*) Parenchyma of tumor.

PLATE XVI., FIG. 7. — Colloid or resting phase — complete involution of simple adenoma. x 90.

(*a*) Capsule.
(*b*) Parenchyma of tumor.

FIG. 8. — Growing or actively hyperplastic stage ot intermediate adenoma. x 90.

(*a*) Non-tumor thyroid.
(*b*) Capsule.
(*c*) Parenchyma of tumor.

FIG. 9. — Involutionary stage of intermediate adenoma. x 75.

(*a*) Non-tumor thyroid.
(*b*) Capsule.
(*c*) Parenchyma of tumor.

Fig. 10a. — Colloid or resting stage of intermediate adenoma, slight edema, and early hyaline degeneration of stroma. x 75.

Fig. 10b. — Colloid or resting stage of intermediate adenoma, more quiescent type. x 75.

Fig. 11. — Growing or actively hyperplastic stage of fetal adenoma. x 75.

(*a*) Non-tumor thyroid.
(*b*) Capsule.
(*c*) Parenchyma of tumor.

Plate XVII., Fig. 12a. — Involutionary stage of fetal adenoma. x 75.

(*a*) Non-tumor tissue.
(*b*) Capsule.
(*c*) Parenchyma of tumor.

Fig. 12b. — Involutionary stage of fetal adenoma (myxomatous adenoma (Wölfler)). x 75.

Fig. 13. — Colloid or resting stage of fetal adenoma. Note variation in size of follicles and follicles containing full colloid content. x 75.

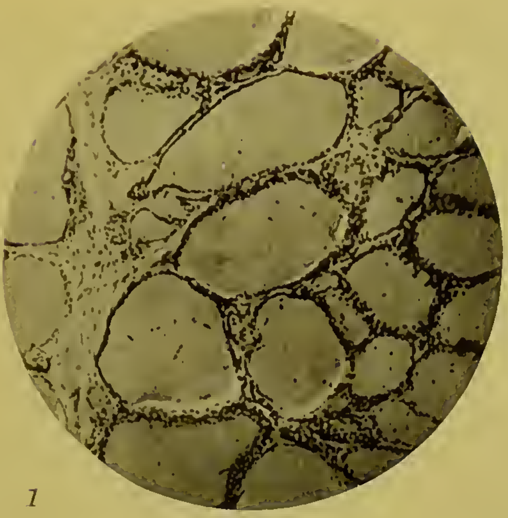
Fig. 14. — Marked hyaline degeneration of stroma with atrophy of follicles from central portion of an involutionary fetal adenoma. x 75.

Fig. 15. — Growing stage of fetal adenoma, x 75, showing:

(*a*) Hemorrhage.
(*b*) Atrophic zone.
(*c*) Zone of closely set follicles.

Fig. 16. — Circumscribed area of thyroid overgrowth in colloid stage from a generally enlarged goitrous gland; not tumor. x 50.

(*a*) Non-tumor thyroid.
(*b*) Adenoma-like formation.



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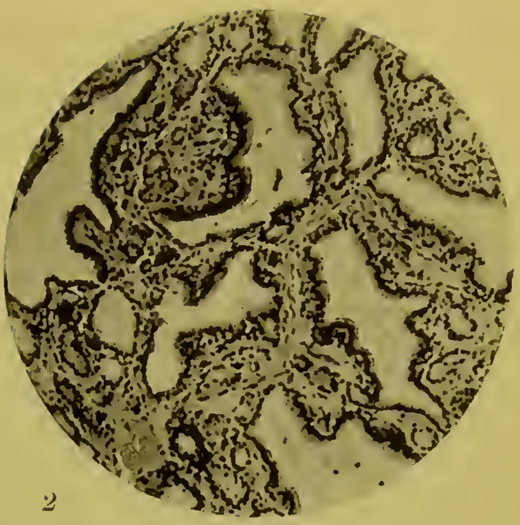

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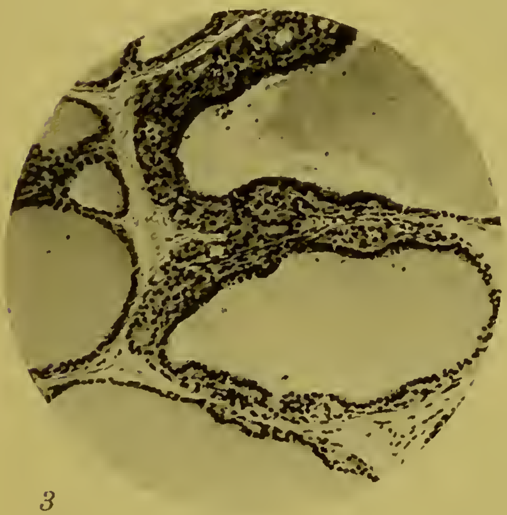

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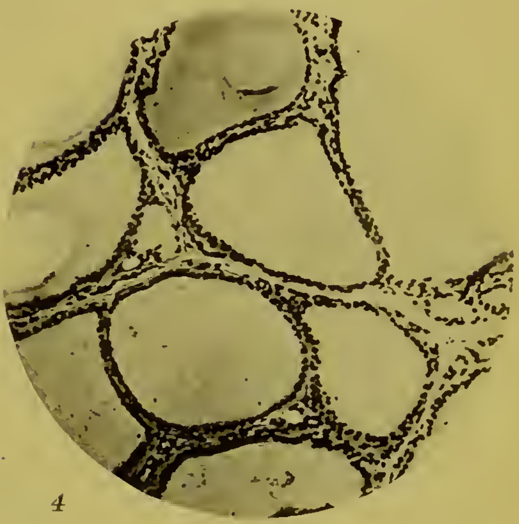

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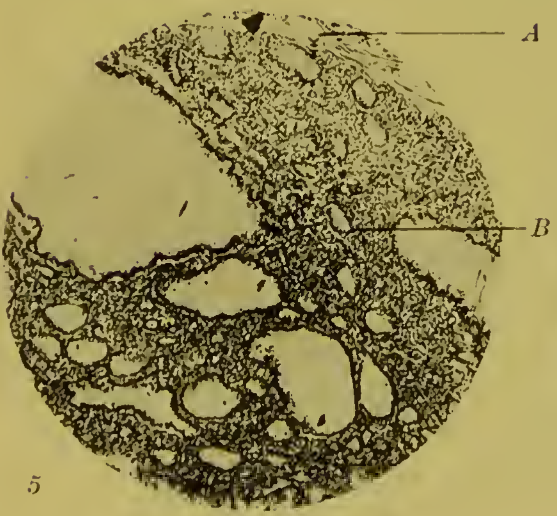

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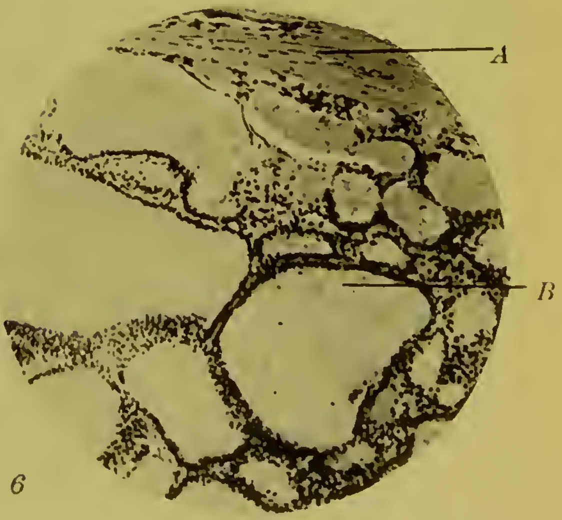

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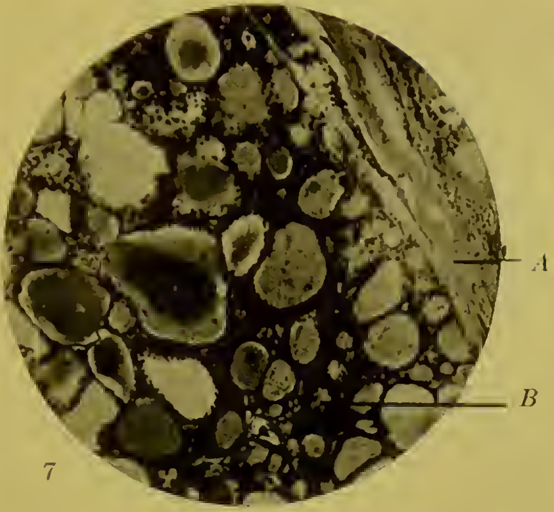

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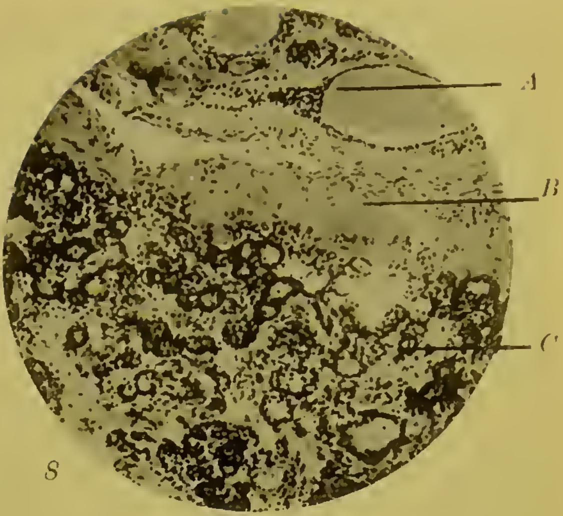

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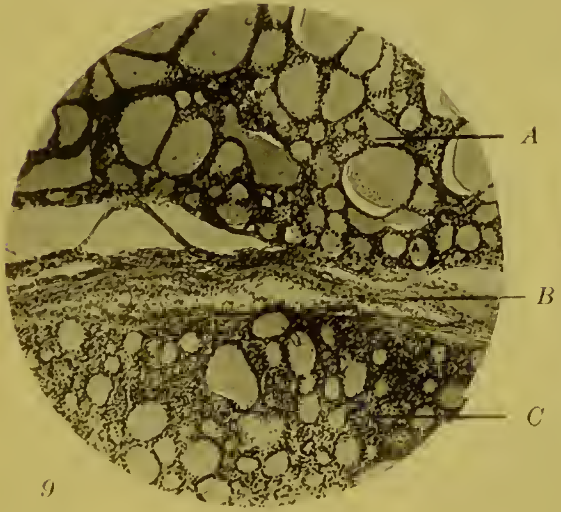

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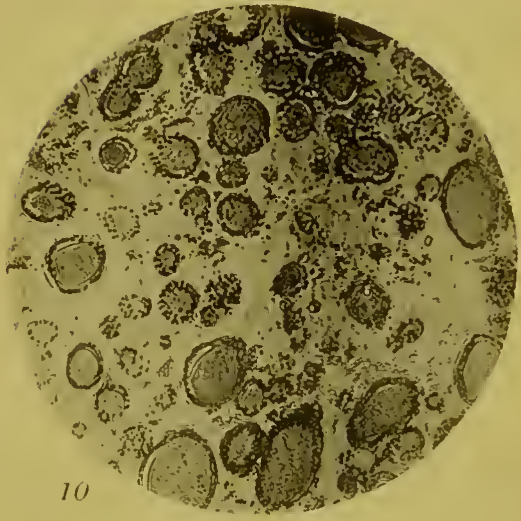

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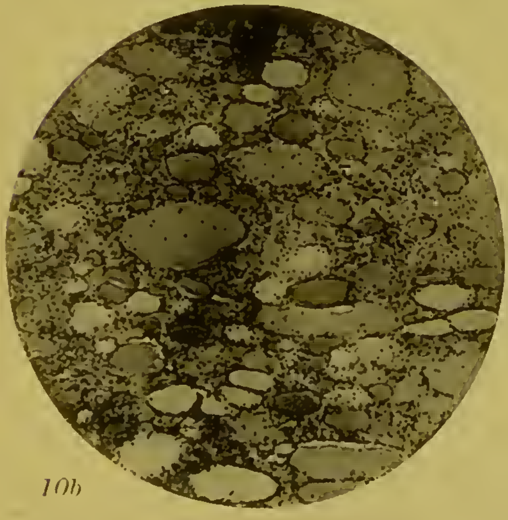

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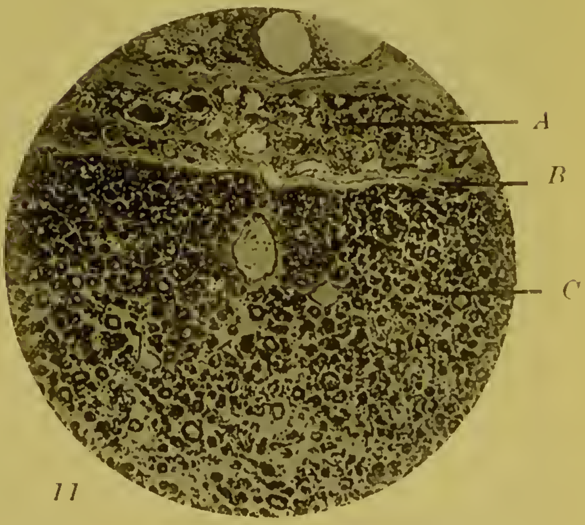

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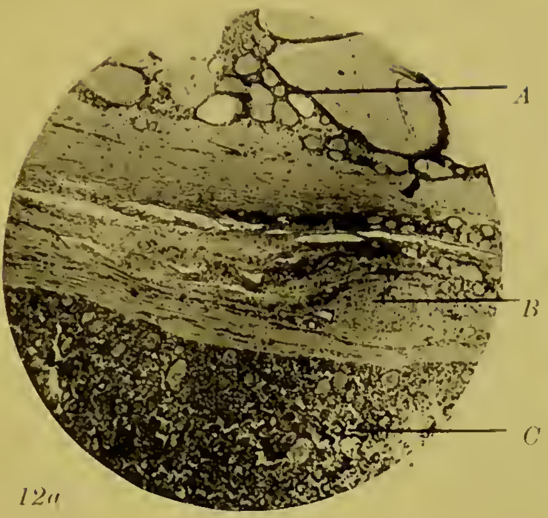

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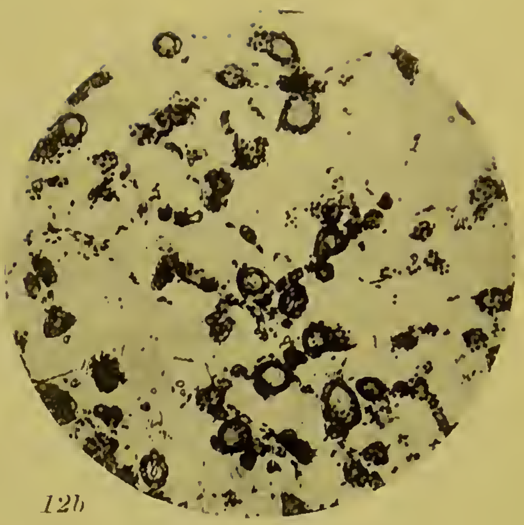

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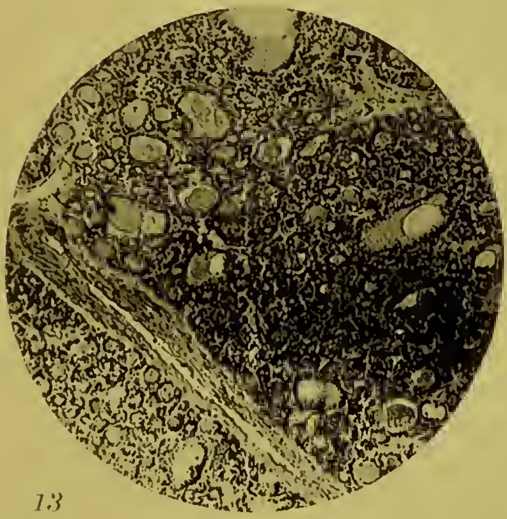

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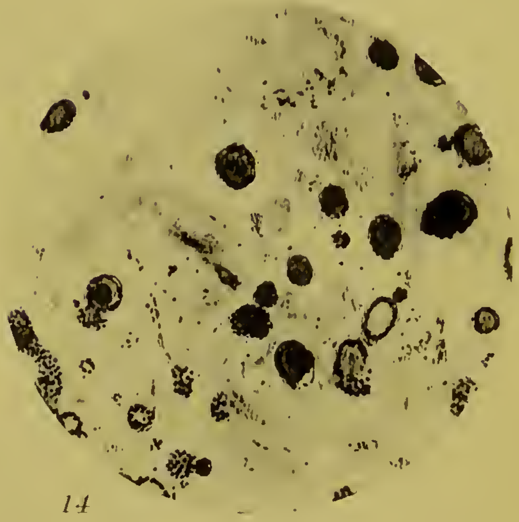

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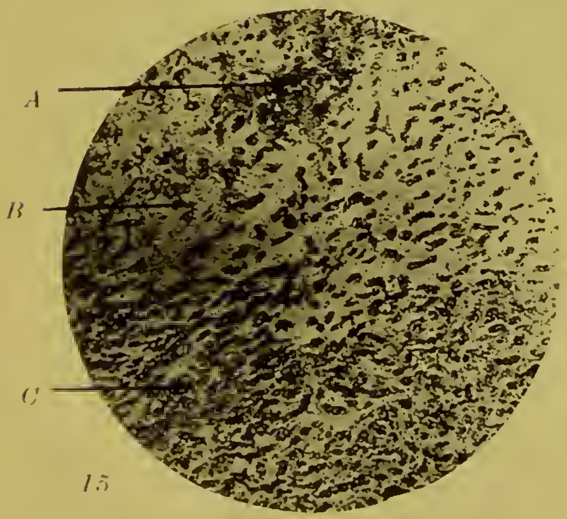

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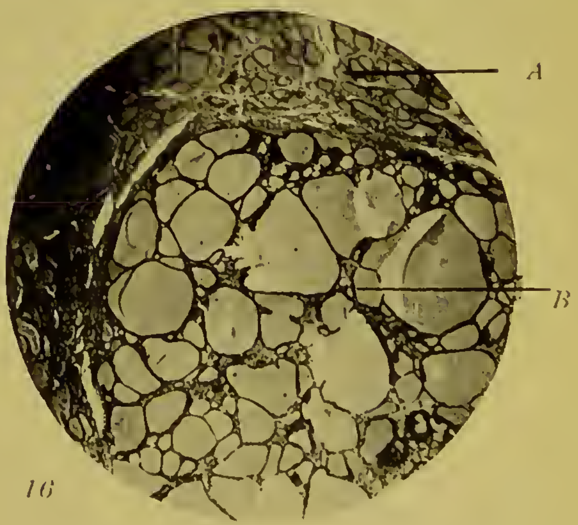

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[From The Johns Hopkins Hospital Bulletin, Vol. XXIV, No. 267, May, 1913.]

# THE EVOLUTION OF THE THYROID GLAND.

By David Marine, M. D.

*(From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University,[1] Cleveland.)*

The thyroid while it does not play an essential rôle in our [135] conception of vertebrates is, nevertheless, one of their most constant and characteristic structures—existing in the same anatomical form from the adult cyclostomes (Petromyzon and Bdellostoma) throughout all the fishes, amphibians, reptiles, birds and mammals.

In the larval Petromyzon—Ammocœtes branchialis—in Amphioxus and in all Tunicates there exists in the ventral midline of the branchial sac a gland-like structure which Huxley[2] first designated as "Endostyle." This endostyle or

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[1] The observations on the Tunicates and Amphioxus were made at the Marine Biological Station at San Diego during the summer of 1911. I am indebted to Dr. W. E. Ritter, Director, for the privileges and excellent facilities of his laboratory. The work with Ammocœtes was done in the Laboratory of Histology and Embryology, of Cornell University, during the summer of 1912. I wish to thank Dr. B. F. Kingsbury for the privileges of his laboratory and many helpful suggestions and criticisms.

[2] Huxley (Observations on Salpa, Pyrosoma, Doliolum, etc., Philos. Trans., 1851, 566) appears to have first introduced the term "Endostyle." He so named it from his belief that this prominent fold in the Tunicates branchial sac was rod-like and served as a brace for the delicate branchial sac. Later work showed it to be a groove lined with highly differentiated gland cells. In German literature it is usually spoken of as the "Hypobranchial Groove." At present both terms are in common use for the structure in both the Tunicates and Amphioxus. When a similar structure in Ammocœtes was found to give rise to the ductless thyroid follicles at metamorphosis the term "Thyroid" was introduced and is now quite generally applied to the endostyle of Ammocœtes. Inasmuch as it has no resemblance to a true thyroid and is entirely similar to the endostyle of the Tunicates and Amphioxus I will use the term "Thyroid" only in connection with the ductless follicle formations.

[135] better, endostylar mechanism[3] has, through the work of Good-
sir, been shown to have the same general structure in both
the Tunicates and Amphioxus, and Wilhelm Müller (Jena-
ische Zeitschrift, 1873, VII, 327), in 1873, pointed out the
similarity of the structure in Ammocœtes with that of the
Tunicates and Amphioxus and suggested that the endostyle of
the lower chordates was homologous with the ductless thyroid
of the higher chordates. This homology has been strength-
ened by the subsequent work of Anton Schneider (Thyroidea
von Ammocœtes, Beiträge z. Vergleich. Anat. u. Entwcklngs-
[136] gesch. der Wirbelthiere, Berlin, 1879, 85) and Anton
Dohrn (Die Thyroidea bei Petromyzon, Amphioxus und den
Tunikaten, Mittheil. Zool. Stat., Neapel, 1886, VI, 49) and is

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[3] In Tunicates in addition to the glandular portion of the endostyle or hypobranchial groove there are two anterior peripharyngeal ciliated bands which begin at the anterior end of the glandular portion of the endostyle, arch around the anterior end of the branchial sac—one on each side—to join in the dorsal midline in the ciliated dorsal ridge or lamina which extends directly posterior to the entrance into the œsophagus. At the posterior end of the hypobranchial groove the marginal folds form two slight ridges which have been traced around the posterior end of the branchial sac to join the dorsal lamina near its termination in the œsophagus.

In Amphioxus the two peripharyngeal grooves also start from the anterior termination of the glandular portion of the endostyle and arch around the anterior border of the branchial sac to meet in the dorsal midline in the dorsal groove (a ridge in Tunicates) which extends back to the entrance to the œsophagus.

In Ammocœtes the grooves are more prominent owing to the fact that the glandular portion of the endostyle is largely cut off from the pharynx and opens into it by a large duct-like elliptical opening. Beginning at the anterior lip of this duct and continuous with it is a correspondingly large, deep, ciliated groove (T-shaped in cross-section) which after extending a short distance anteriorly branches into right and left ciliated peripharyngeal grooves which arch around the anterior end of the pharynx to join the prominent midline dorsal ridge where they fuse with the ciliated epithelium on the sides of this pendant-like fold and continue back to the œsophagus. The ventral or most dependent portion of this fold is covered with stratified squamous epithelium out of which the permanent œsophagus will be formed. Beginning at the posterior lip of the endostyle duct and continuous with it is a midline, shallow, ciliated groove which extends back to the entrance to the larval œsophagus.

as firmly founded as morphological studies make possible. [136] Below the chordates nothing comparable to an endostylar structure has been made out and even in the lowest class of chordates—Balanoglossus—the structure is absent. Ritter (On a new Balanoglossus larva from the coast of California and its possession of an endostyle, Zool. Anz., 1894, XVII, 24) has described a ridge or band of high ciliated epithelium in the midline of the œsophagus of a Tornaria (larval Balanoglossus) which, from its location and from the generally accepted systemic position of Balanoglossus he suggested might be homologous with the Tunicates endostyle. Morgan (The Development of Balanoglossus, Jour. of Morph., 1894, IX, 1) also described this structure but did not use it as evidence of this animal's possible relation to the true chordates. While I was working in Dr. Ritter's laboratory during the summer of 1911 the question of the relation of this oro-œsophageal mechanism of Tornaria to the Tunicate endostyle was discussed with him and he expressed the opinion that the evidence was insufficient for assuming any phylogenetic relationship.

Leaving out Balanoglossus as doubtful possibly as regards its systemic position as a chordate and certainly as regards the endostylar nature of the oro-œsophageal, ciliated, gland cell mechanism, the lowest undoubted and living relative of the thyroid mechanism is found in the Tunicates.[4]

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[4] The endostyle-thyroid mechanism has been utilized in the philosophical discussions of the origin of vertebrates. Whether the primitive Tunicate which Brooks (The Genus Salpa—Johns Hopkins Press, Baltimore, 1893) believed was typified in all essentials in the existing *Appendicularia* arose in the main stem of the descent of chordates or whether all Tunicates are degenerate forms of fish-like ancestors unrelated to the vertebrate tree, as Dohrn and others believe, is a question that does not concern us here. The data of the endostyle-thyroid mechanism can only be a minor item in such a controversy, but so far as they can be utilized in the elaboration of any hypothesis of the origin of vertebrates it seems that the views of Brooks more nearly harmonize these facts of structure, function and historical sequence than do the views of Dohrn, Gaskell, Patten and others which would leave the origin of the endostyle unexplained. Brooks (Origin of the Thyroid, Johns Hopkins Hosp. Bull., 1893, IV, 47) considered the endostyle of the Tunicate to have arisen with the branchial sac as a specialization to meet the needs of its habits and modes of life.

[136] More than a thousand species of Tunicates have been described (Ritter, W. E.: Am. Naturalist, 1907, XLI, 453). The several groups present great variations in external appearance (sessile, pellagic, simple, colonial) although their internal structure is strikingly constant. The endostylar mechanism is a good example of this constancy of organs and it rarely presents variations in morphology sufficient to be of value in the differentiation of species (Herdman: Liverpool Marine Biol. Mem., 1899, XI, No. 1, 14).

The large simple Ascidian Holocynthia johnsonii, is very common in San Diego Bay and most of my observations were made on this species, although through the courtesy of Dr. Ritter I made microscopic preparations from many of his general collection of Tunicates including several species of Salpa, Pyrosoma, Ciona, Cynthia, Doliolum and Appendicularia.

## TUNICATES.

(FIG. 1, PLATE III, AND FIG. 2.)

The glandular portion of the endostylar mechanism forms a groove (roughly U-shaped in cross-section) lying in the ventral midline of the pharyngeal or branchial sac. This groove begins near the anterior end of the pharynx just posterior to the oral tentacles, at the ventral junction of the

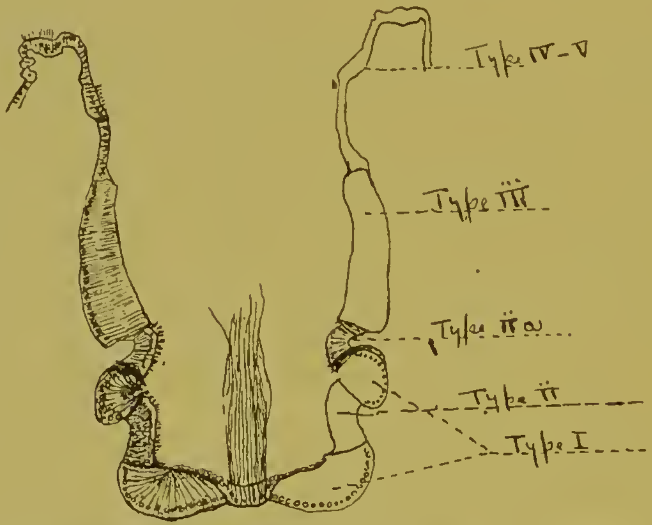


FIG. 2.—Diagrammatic outline of endostyle of Holocynthia johnsonii as seen in cross-section.

peripharyngeal ciliated bands and extends backward nearly to [136] the œsophagus. In all the larger Tunicates it is visible to the naked eye as a prominent, whitish, longitudinal fold embedded in the wall of the branchial sac. The groove opens throughout its length into the branchial sac but owing to the elasticity of the branchial sac and the muscular mantle the mechanism exists for closing the groove by apposing its lateral walls.

Histologically the groove is lined with highly differentiated gland cells. When seen in cross-section there is in the ventral midline of the groove a tract composed of closely packed columnar cells with deeply staining nuclei bearing whiplike cilia whose lengths in many species nearly equal the depth of the groove. On either side of this unpaired tract of ciliated cells the fundus, lateral wall and marginal fold of the groove [137] contain symmetrical epithelial formations. These symmetrical epithelial tracts form the basis of homology with the endostyle of Amphioxus and Ammocœtes. There are at least four and possibly five types of epithelia. The most uniformly constant type in form and position comprises two large tracts of cuneiform gland cells in each half of the endostyle which I will call Type I. These wedge-shaped cells of each tract are arranged radially from the common point of opening into the endostylar groove and each bundle is roughly fan-shaped. The more ventral bundle is usually slightly larger. The cells are pale, granular, with large vesicular nuclei lying at the bases of the cells (periphery of the bundle). These two bundles of Type I cells in each half are separated by an intervening layer of short, closely packed, columnar, ciliated cells with deeply staining nuclei lying in different levels of the cell bodies which may be designated as Type II. The height and extent of these cells vary with different species being as a rule more extensive in the Salpae. Just above the upper bundles of cuneiform cells (Type I) and closely applied to them is another short area of cells, columnar and ciliated in Ascidians and cuboidal in Salpæ, which differ from the cells of Type II. For convenience these may be spoken of as Type II-A. Above this somewhat variable layer of Type II-A cells is a very long area in each half of large, high, columnar cells that may be designated as Type III. These cells are not ciliated

[137] in any of the species examined. Above this group in each half and continuing to the marginal fold or lips of the groove is another group which I shall designate as Types IV and V. These cells are in general cuboidal, often containing yellow pigment granules, and non-ciliated although, just at the margin of the fold and its junction with the branchial mucosa, there is on each side a small area of ciliated, low, columnar cells with deeply staining nuclei.

Summing up then, the endostylar groove proper is lined with highly differentiated epithelium which may be divided into at least four and possibly five different types in addition to the unpaired midline epithelium.

## AMPHIOXUS LANCEOLATUS.

(FIG. 3, PLATE III, AND FIG. 4.)

The glandular portion of the endostyle in the adult Amphioxus forms a broad shallow groove situated in the ventral midline of the pharynx and extending practically the whole length of the pharynx terminating anteriorly in the peripharyngeal ciliated bands and posteriorly just anterior to the œsophagus. The groove opens into the branchial sac throughout its length and it does not appear that the groove can be closed off from the pharynx as it can be in the Tunicates.

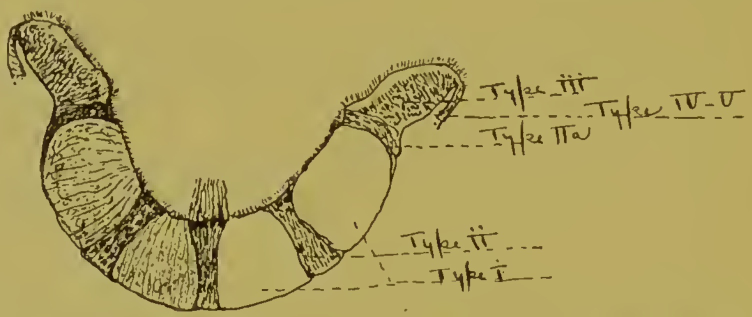


FIG. 4.—Diagrammatic outline of cross-section of endostyle of Amphioxus calif.

Histologically as Goodsir, Müller, Schneider, Dohrn and many others have shown it is composed of the same basic types of epithelium already described for the Tunicates. Seen in cross-section there is in the ventral midline of the endostyle a small bundle of tall, columnar, deeply staining cells with long upright cilia. On either side of this unpaired tract [137] are two bundles of large, pale, columnar, slightly wedge-shaped cells, which, on account of their resemblance to Type I of the Tunicates, may also be designated as Type I. They differ from the corresponding cells of the Tunicates in that they are tipped with short cilia-like processes. Between the two bundles of cells of Type I on either side is a very narrow layer of columnar ciliated cells with deeply staining nuclei which from their location and staining characteristics are similar to Type II of the Tunicates and may here be designated as Type II. Just above and closely applied to the upper bundle of cuneiform cells of Type I on each side is a very narrow zone of cells which, as regards size, shape and staining reaction, are almost identical with Type II and I have designated them Type II-A since they correspond in position to the group of cells designated Type II-A for the Tunicates. Beyond or above the cells of Type II-A and producing the marginal fold are long areas of high columnar, ciliated, closely packed cells with deeply staining nuclei which, as regards extent and location, closely resemble the Type III for the Tunicate endostyle except that they are ciliated and may also be designated as Type III for the Amphioxus. Above the cells of Type III and curving sharply externally and ventrally and fusing with the branchial mucosa in the ventral-lateral sulcus of the pharynx is a poorly defined layer of low cuboidal epithelium which is analogous with Types IV and V of the Tunicates in position and structure and may also be designated Types IV and V.

To sum up then, the endostylar groove proper is lined by highly differentiated epithelia of at least four different types in addition to the median, unpaired, ciliated tract and is similar in all its general characteristics to the endostyle of Tunicates. There are some differences. Thus the groove in Amphioxus is more compact, shallower and relatively wider than in Tunicates. Only three of the types of epithelium are contained in the groove proper in Amphioxus and all these cells are ciliated and of quite uniform height. These differences are but modifications as the fundamental structures correspond with those of the Tunicates.

## [137] AMMOCŒTES BRANCHIALIS.

(FIGS. 5 AND 6, PLATE III.)

The fully developed endostylar groove shows many modifications of the form seen in the Tunicates and Amphioxus. Thus the glandular portion is shut off from the pharynx except for the large elliptical duct-like orifice, and the pharyngeal grooves anteriorly and posteriorly are correspondingly
[138] increased. Embryologically it has been shown (Reese, A. M.: Proc. Am. Acad. Nat. Sci., Phila., 1902, 85) that at first the endostyle is an open ventral midline groove in the floor of the branchial sac just as in Tunicates and Amphioxus but in the course of its development great distortions and modifications are required.

The glandular portion of the endostyle is divided into symmetrical right and left halves by the median septum and each half contains the same complement of epithelia that is present in the whole endostyle of the Tunicates or Amphioxus, or in other words—the fully developed endostyle of Ammocœtes is similar to two simple endostyles placed side by side. In cross-section of a half of the endostyle one notes the absence of the tract of cells which in Tunicates and Amphioxus bears the long whiplike cilia as well as of any tract of cells that may be homologized with them. The next group of cells consists of the four tracts—two on each side of the midline of each half—of large cuneiform cells which in shape, size and staining reactions are identical with Type I of the Amphioxus and Tunicate endostyle. These cells formed the basis for the first homology by Müller. On each side of the openings of these bundles of wedge-shaped cells are areas of columnar, ciliated, closely set cells with deeply staining nuclei that may be designated as Type II since they correspond in location and type to the Type II of Amphioxus and Tunicates. Closely applied to one border of the cells of Type II is another group of larger, columnar, ciliated cells with large vesicular nuclei and containing yellow pigment granules which I have designated Type III. In the Tunicate endostyle the yellow pigment is commonly present in the cells which have been grouped under Types IV and V. It may be, therefore, that the epithelium grouped as Type III in the Ammocœtes endostyle is

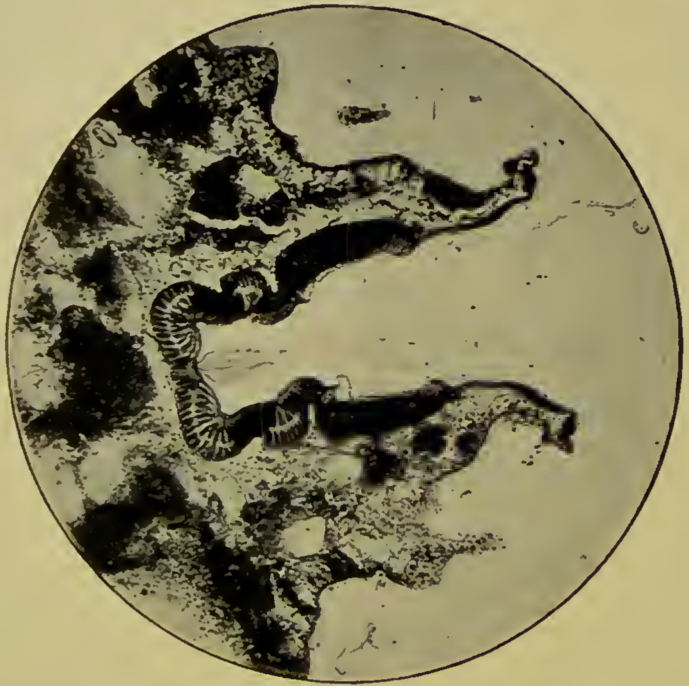
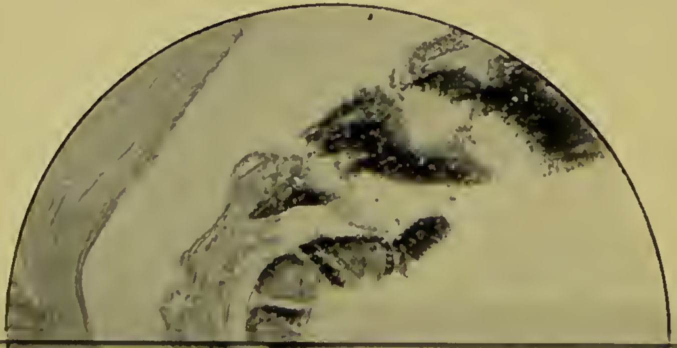
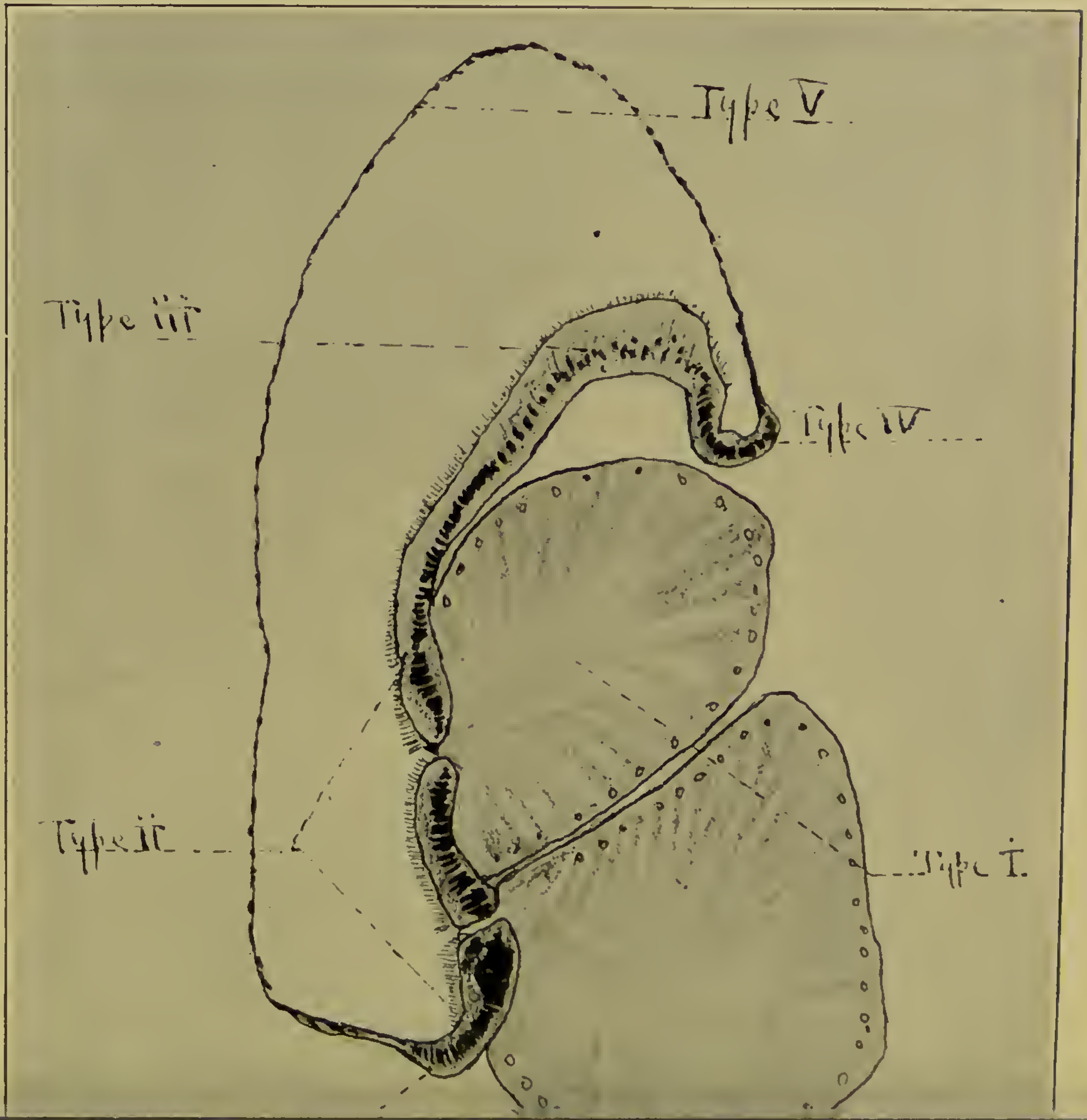


FIG. 1.—Photomicrograph of cross-section of endostyle of Holocynthia johnsonii with adjacent parts of branchial sac.





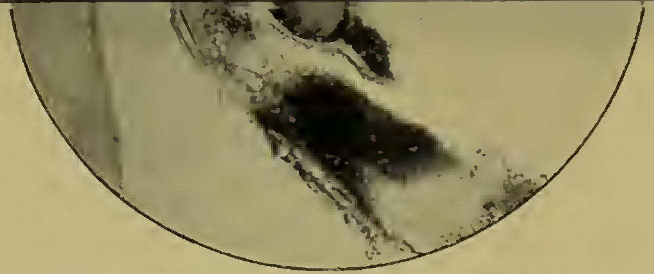


FIG. 3.—Photomicrograph of cross-section of endostyle of Amphioxus calif., showing relation to branchial sac and gill filaments.

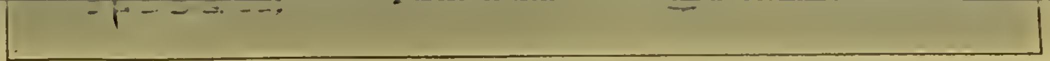


FIG. 6.—Camera outline of cross-section of the left external chamber of the endostyle of Ammocœtes posterior to the coiled inner chambers, *i. e.*, containing ¼ the epithelial investment of the whole endostyle or ½ the epithelial investment of the simple endostyle of Tunicates or Amphioxus.

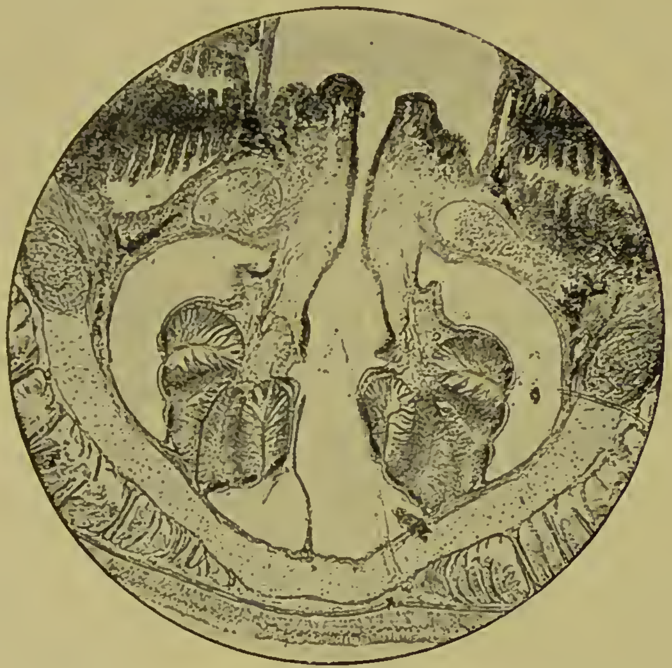


FIG. 5.—Photomicrograph of endostyle of Ammocœtes branchialis as seen in cross-section at the level of the duct. One notes twice the epithelial complement of the simple endostyle of Amphioxus or Tunicates.

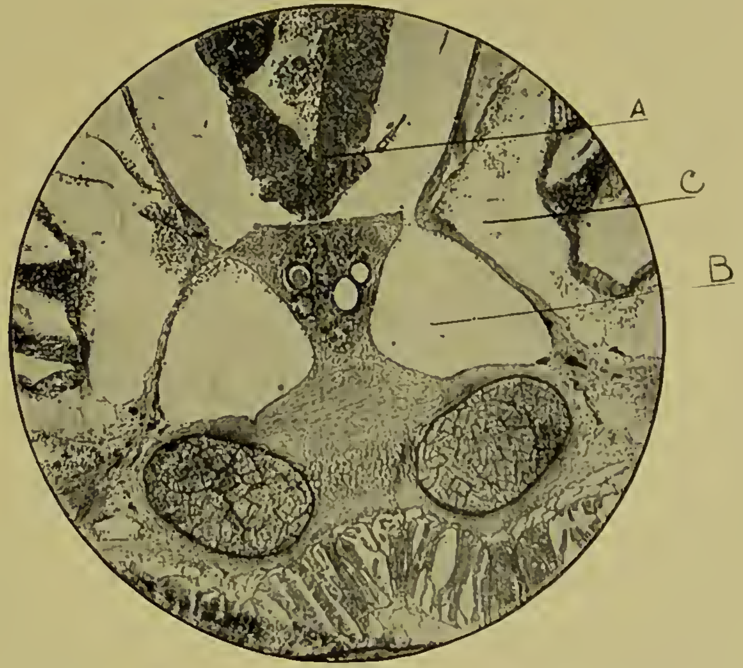


FIG. 7.—Photomicrograph of cross-section of thyroid area in a recently metamorphosed Ammocœtes branchialis showing relation of follicles to (A) tongue, (B) blood spaces, (C) gills.

actually homologous with Types IV and V of Tunicates rather than with the epithelium I have grouped as Type III. Beyond Type III, covering the apex of the visceral invagination and the baso-lateral angles and continuous with the epithelium lining the duct and pharyngeal groove, is another type which in Ammocœtes I have grouped separately as Type IV. As will be seen later this Type IV is the most important epithelium concerned in the formation of the ductless thyroid follicles at metamorphosis. Type IV merges into another form which lines the parietal wall of the chamber and is designated as Type V. The cells of this type are flat, almost endothelial in form. [138]

The metamorphosis (Fig. 7, Plate III) of Ammocœtes into Petromyzon which was first made known in 1856 by A. Müller gives us the great connecting link in the evolution of the thyroid mechanism, without which the relation of the endostyle to the thyroid could not have been known. The occurrence of a living animal which in its larval form has an endostyle only and in its adult form a ductless thyroid only allows one to observe the formation of typical ductless thyroid follicles out of the fullest development of the endostyle. Only the endostyle of larval Cyclostomes undergoes this metamorphosis. The metamorphosis from its beginning to its completion in Ammocœtes extends over a period of at least a month. The endostylar changes are at first a general shrinkage in all the component parts of the organ. The changes of greatest significance are those of the epithelia. The first to disappear are the tracts of large cuneiform cells (Type I) which are the most characteristic cell groups in the endostyle. The cells of Type III stand next to those of Type I in point of the rapidity of the cell atrophy and absorption although remnants of this type (easily identified by means of the yellow pigment) can be seen after the ductless follicles are formed. The flattened epithelial cells of Type V show the next most extensive changes although here also atrophic and distorted cell masses may be preserved until ductless follicle formation is nearing completion. It seems certain that the cells of Types I, III and V take no part in the ductless follicle formation. With the Types II and IV one cannot be certain that both do

[138] not take part although Type IV is certainly the most important epithelium persisting as the lining epithelium of the permanent follicles. The fact that Type IV is the predominant persistent epithelium of the ductless follicles and also that it is continuous with and probably identical with that epithelium lining the gland duct and the ventral pharyngeal grooves seems to me of the greatest significance in the interpretation of the origin of the thyroid of higher vertebrates. This observation, taken in connection with the observations of Dohrn that in the torpedo embryo transient rudimentary pharyngeal grooves are present, is strong evidence that the ductless thyroid of higher vertebrates originates from a pharyngeal anlage closely related to if not homologous with the epithelium which lines the pharyngeal groove and duct of the Ammocœtes endostyle.

The ductless thyroid follicle of the type present in Petromyzon persists throughout all the remaining classes of vertebrates in the same anatomical form and this allows some conception of the vast antiquity of the gland. In none of the chordates above the larval Cyclostomes is there a true endostyle formation. Dohrn and others have shown that a transient and rudimentary formation of the pharyngeal grooves can be made out in the very early stages of development of the lower rays and sharks but even this remnant of the endostylar mechanism cannot be traced into the higher vertebrates.

Beginning with the fishes and continuing throughout the remaining vertebrates the problem of the evolution of the thyroid is the problem of the embryology, which is still unsettled as regards mammals. In fishes the thyroid resembles very closely that of Petromyzon in position, arrangement of follicles, and as a rule (excepting elasmobranchii) in the absence of a capsule. The gland in fishes arises from a single median ventral anlage of entoderm in the region of the first gill arch.

In amphibians the adult thyroid is paired. Each body is surrounded by a fibrous capsule. Embryologically the gland arises from a single median ventral anlage of entoderm in the region of the first gill arch. This anlage grows downward and backward and divides into right and left halves which in

the adult frog come to lie on the ventral surface of the pos- [138]
terior horns of the hyoid bones (Mayer: Zur Lehre von der
Schilddrüse und Thymus bei den Amphibien, Anat. Anz., [139]
1888, III, 97).

In reptiles the adult thyroid is unpaired in the snakes (ophidia) and turtles (chelonia) and paired in the other groups (lizards, crocodiles, etc.) and lies in the thoracic cavity just above the pericardium. Embryologically all observers are in agreement that it arises from a single median ventral anlage in the region of the first gill arch and is shifted backward with the aortic arteries.

In birds the thyroid lobes are separate, oval bodies placed on each side of the vertebral column a short distance above the pericardium and usually partially imbedded in the lowermost lobules of the thymus. Embryologically the first accurate description was given by Remak in 1855 who derived the gland from an unpaired median ventral anlage of pharyngeal entoderm in the region of the first gill arch. He observed that as development proceeded it divided into a T-shaped process and shifted backward with the aortic arches to occupy an intra-thoracic position. The thyreo-glossal tract and isthmus were absorbed. These observations were accepted (by Gotte, Müller and Seessel) until 1881 when Stieda and Wölfler stated that the thyroid had a lateral origin as well as a median. Mall, in 1887 (Archiv. f. Anat. u. Physiol., 1887, 1-34), working with the development of the chick thyroid came to the conclusion of Remak that the chick thyroid is derived wholly from the single median anlage in the region of the first gill arch. The derivatives of the fourth gill pouch Mall designated as “Körpern Y” and concluded that they had nothing to do with the thyroid. Subsequent work has established the original views of Remak, Müller and Mall, that the avian thyroid is derived solely from the single, median, ventral, pharyngeal anlage of the first aortic arch.

In mammals the question as to whether the thyroid arises from one or three anlagen is still argued although the evidence brought forward during the last ten years has apparently settled the controversy. In mammals only, therefore, is there at present any doubt as to the single and median origin of the

[139] thyroid. Many of the earlier observers including Wilhelm Müller (Jenaische Zeitschrift, 1871, VI, 428) believed the mammalian thyroid arose from the single median anlage. Although Remak in the chick, His and Kölliker in the rabbit, Wölfler and others had partially observed paired lateral bodies whose fate could not be decided but which some believed to be part of the thyroid tissue, it remained for Stieda, in 1881, working with sheep embryos, to give the first important description of these paired bodies from the fourth pouch and he called them "Lateral thyroid anlagen." He believed the whole thyroid was derived from these two lateral anlagen. In 1883 Born (Archiv. f. mikr. Anat., 1883, XXII, 271), working with pig embryos confirmed Stieda's discovery of the lateral thyroid anlagen, but showed that the median anlage formed thyroid tissue and fused with the two lateral anlagen thus deriving the thyroid from three separate anlagen. His in 1885 (Anatomie menschlicher Embryonen, Leipzig, 1885, Heft 3), working with human embryos also found these lateral thyroid anlagen arising from the fourth pouch and concluded that the median anlage formed the isthmus and pyramidal process of the thyroid while the lateral anlagen formed the lateral lobes. This view, while it prevailed for 20 years following 1885, was questioned by Kastschenko, in 1887 (Archiv. f. mikr. Anat., 1887, XXX, 1), who worked with the sheep embryo. He concluded that these lateral anlagen played no important rôle in the development of the thyroid. These same bodies in the chick Mall had designated "Körpern Y." Van Bemmelen, in 1889 (Anat. Anz., 1889, IV, 400), homologized the "Körpern Y" (Mall) of chicks, post-branchial bodies (Maurer) of amphibians and the lateral thyroid anlagen (Stieda, Born) of mammals with the superpericardial bodies (Van Bemmelen) of Selachians and Ganoids. The later work of Maurer (Die Schlundspaltenderivative von Echidna, Verhandl. d. Anat. Gesellsch., 1899, XIII, 88) of Hermann and Verdun (Compt. rend. Soc. Biol., 1900, LII, 933) have further established that the lateral thyroid anlagen or post-branchial bodies play no part in the development of the thyroid, that in the lower mammals (Monotremes and Marsupials) they tend to remain separate from the thyroid

proper, although in the placental mammals they are as a rule [139] imbedded in the posterior lateral portion of the lateral thyroid lobes. Fox (Am. Jour. Anat., 1908, VIII, 187) arrived at similar conclusions for the rabbit embryo. Grosser (Anat. Anz., 1910, XXXVII, 337) has summarized this trend in the views as to the fate of the lateral thyroid anlagen. He would abandon the term "Lateral Thyroid Anlagen" as a misnomer and would substitute the terms "Ultimobranchial" or "Telobranchial" body instead of post-branchial body (Maurer) or superpericardial body (Van Bemmelen) because it is believed that in the human embryo these bodies are derived from the

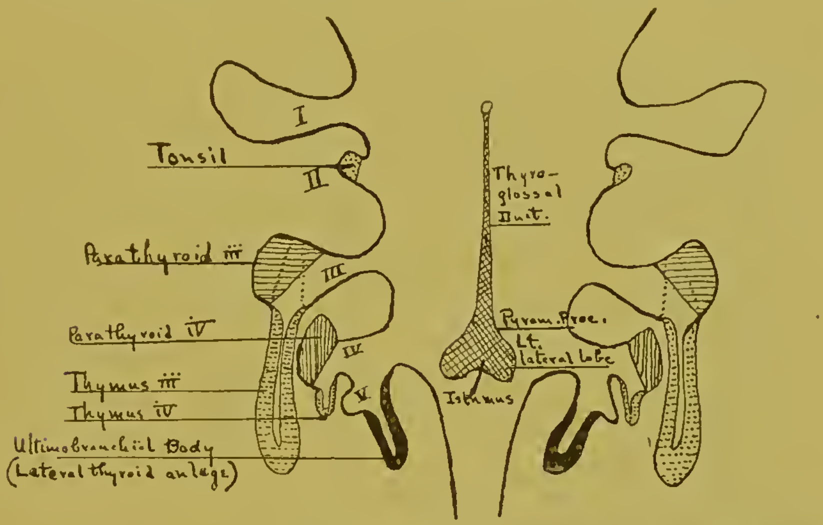


FIG. 8.—Schema of the branchiogenic derivatives in man (after Grosser in Keibel and Mall—Human Embryology, 1912, II, 461).

rudimentary fifth pouch rather than from the fourth. (Fig. 8.)

With this short review of the embryological evidence that the mammalian thyroid is derived wholly from the median anlage I shall next review briefly the equally important evidence obtained from the developmental anomalies and the pathology all of which is associated with the median anlage. It has long been known that thyroid tissue in the form of a tract or as isolated masses was not of uncommon occurrence along the path of descent of the original thyroid anlage, *i. e.* [140] from the foramen cæcum to the thyroid isthmus (Merten: Archiv f. Anat. u. Physiol., Anat. Abth., 1879, 483). Streck-

[140] ciscn (Virchow's Archiv, 1886, CIII, 131 and 215), in a careful series of observations on the human thyroid, showed that the persistence of thyroid tissue along the thyreo-glossal tract was very common in districts where goitre was endemic and was always associated with a general thyroid enlargement which probably began during fœtal life. His (Archiv f. Anat. u. Physiol., Anat. Abth., 1891, 26) pointed out that in man the thyroid tract begins to atrophy normally during the fifth week of intra-uterine life. When abnormal demands for thyroid activity begin before this normal atrophy is completed a cell proliferation involving all the existing thyroid tissue takes place instead of the physiological resorption. The so-called sub-lingual, suprahyoid and infrahyoid thyroid masses as well as the pyramidal process are the commoner persistent forms of the thyreo-glossal tract. Thyreo-glossal cysts in which all thyroid tissue have disappeared are also relatively common in goitre districts. While much less studied, similar evidences of persistent remnants of the thyreo-glossal tract are present in the lower mammals. Wölfler has described such cases. I have also seen many instances in congenital goitre in dogs. Here the most common forms are the subhyoid accessory thyroids and the persistence of the thyroid isthmus. Goodcy (Anat. Anz., 1910, XXXVI, 104) has described a persistent canal of His (thyreo-glossal duct) in a primitive selachian (Chlamydoselachus Anguincus).

In cases of aplasia of the thyroid Maresch (Zeitschr. f. Heilkunde, 1898, XIX, 249) and Erdheim (Zeigler's Beitr. z. Path. Anat. u. Allg. Path., 1904, XXXV, 366) have shown that the parathyroids are usually normal but that in the region of the fourth pouch parathyroid a cystic entodermal rest corresponding to the post-branchial or ultimo-branchial body or lateral thyroid anlage may usually be found although never any thyroid tissue. Getsowa (Virchow's Archiv, 1911, CCV, 208) working with the atrophic goitrous thyroids of cretins and idiots has observed these cell rests and cysts between the fourth pouch parathyroid and the lateral thyroid lobes. She noted that these rests did not react with the thyroid in developing goitre and considered them as ultimobranchial bodies, which in mammals are functionless remnants [140] or at least take no part in the formation of thyroid tissue.

All the evidence, therefore, from embryology, from congenital developmental defects, and from pathology is against the view that the post-branchial bodies or "Körpern Y" or lateral thyroid anlagen have any part in the formation of the thyroid gland. The different conclusions of the earlier observers were probably due in part at least to the fact that in mammals these bodies fused with the lateral thyroid lobes although in all lower vertebrates they remained separate and while functionless in mammals are believed to be active organs in sharks and possibly amphibians.

## PHYSIOLOGY.

Concerning the function of the endostyle organ little is known. The observations and experiments of Giard (Arch. de Zool. Exper., 1872, I, 525 and 1873, II) and Fol (Morph. Jahrb., 1874, I, 222) on the transparent Synascidia, Salpa and Doliolum by means of India ink have shown that the endostyle is an active gland concerned in an important way with the collection of and possibly digestion of food. They observed that the gland cells secrete a slime or mucinous substance in which the food particles taken into the branchial sac with the respired water are caught up and that this mucin is formed into a cord by means of the cilia and carried forward to the peripharyngeal bands, thence along these ciliated tracts to the dorsal lamina and thence backward to the œsophagus. These observations have been confirmed by subsequent observers. All observers have suggested a possible chemical digestive action for this mucin but of this possibility nothing is known.

Reasoning from analogy the same function is believed to obtain in the Amphioxus and Ammocœtes endostyles. Alcock (Jour. Anat. and Physiol., 1899, XXXIII, 612), working under Gaskell, studied the digestive effects of extracts of various Ammocœtes tissues on fibrin. She was able to demonstrate a pepsin-like ferment in the liver, intestine and pharynx, but could obtain no evidence that the endostyle contained any proteolytic ferment.

In view of the important relation of iodin to the ductless

[140] thyroid I have made several iodin determinations on the tissues of Holocynthia johnsonii, on Salpa confederata and Amphioxus the averages of which are as follows:

| | | Mgm. of iodin per Gm. dried tissue. |
|---|---|---|
| Salpa confederata (whole animal) | | Trace |
| Holocynthia johnsonii | (whole animal except tunic) | 0.03 |
| " " | tunic tissue | 3.63 |
| " " | branchial sac tissue | 0.00 |
| " " | endostyle tissue | 0.00 |
| " " | dorsal lamina tissue | 0.00 |
| " " | mantle (muscular tissue) | 0.01 |
| " " | gut (with contents) | 0.08 |
| " " | liver tissue | 0.03 |
| " " | ova | 0.01 |
| Octopus—legs | | 0.00 |
| Amphioxus lanceolatus (whole animal) | | Trace |

The results are wholly negative as regards the endostyle but one thing of interest, which so far as I have seen is not referred to, is the very high iodin content of the tunic of the Ascidians. The tunic contains much cellulose and the presence of iodin in it may be of the same significance as in ocean plant life generally.

As to Ammocœtes, I have kept them for nine months in an atmosphere of iodin to see whether the structure of the endostyle might be modified by it. The results were entirely negative. Iodin determinations were not made on the endostyle tissue either with or without administration of iodin. From these meager observations on the physiology of the endostyle one can state only what morphologists had already stated, that the function of the endostyle is probably quite different from that of the ductless thyroid. From its size and position at the beginning of the alimentary tract of all the three classes of animals possessing the endostyle and from its known mechanical action in the collection of food particles by
[141] means of its mucinous secretions it certainly subserves a function of prime importance in the nutrition of these animals.

## SUMMARY.

Tunicates, Amphioxus and Ammocœtes are the only classes of animals which possess well developed endostyles. Goodsir, Müller, Schneider, Dohrn and all subsequent observers have shown that morphologically the endostyles are fundamentally [141] identical in all. Cyclostomes, fishes, amphibians, reptiles, birds and mammals are the only classes of animals which possess ductless thyroids, the follicles of which are anatomically identical in all. The demonstration of the ontogenetic relationship between the endostylar mechanism and the true thyroid has been made possible through the preservation of a single class of chordates—the Cyclostomes—and the discovery by Schneider that during the metamorphosis of Ammocœtes into Petromyzon, typical ductless thyroid follicles are formed from the endostyle and as I have shown (Jour. Exp. Med., 1913, XVII, No. 4) out of one and possibly two persistent epithelia. The most important of the epithelia concerned in the formation of the ductless follicles is that form which is continuous with the lining epithelium of the duct and pharyngeal grooves. Additional data of this relationship are afforded by the observations of Dohrn that in some of the lower fishes a transient system of peripharyngeal grooves is present in the earlier stages of embryological development. This overlapping, so to speak, of the endostylar mechanism of the lowest chordates onto the ductless thyroid mechanism of the higher chordates is as firmly established as morphological studies make possible. Studies in the embryology of the ductless thyroid have shown that in fishes, amphibians, reptiles and birds the thyroid arises solely from a median, single, ventral downgrowth of the pharyngeal entoderm in or slightly anterior to the first aortic arch. In mammals this symmetry of development was believed to be departed from through the discovery by Stieda of the so-called "lateral thyroid anlagen" from the fourth or more accurately in man the rudimentary fifth gill pouch, but the work in the embryology, in the pathology and in the developmental defects of the thyroid during recent years has shown that these lateral bodies which in mammals only become imbedded in the lateral thyroid lobes take no part in the formation of thyroid gland tissue. This solution of the origin of the mammalian thyroid from the single median anlage harmonizes the location and development of the endostyle with the location and development of the ductless thyroid. The thyroid mechanism, there-

[141] fore, irrespective of the possible phylogenetic relationship to the chordate stem of the several classes of animals concerned, appears to have been evolved through a direct line of descent from the Tunicates through the Amphioxus, fishes, amphibians, reptiles, birds and mammals. The meager evidence of the physiology in both the endostyle and the ductless thyroid gives no suggestion of an inter-relationship or function. Primarily the thyroid is a part of the alimentary tract and in its endostylar form is a digestive gland of great importance through its probable external secretion. In its ductless form it is only the atrophic remnant of its ancestor which, while it has suffered a corresponding distortion of function, still profoundly influences the animal's nutrition through the effect of its probable internal secretion.

# THE ANATOMIC AND PHYSIOLOGIC EFFECTS OF IODIN ON THE THYROID GLAND OF EXOPHTHALMIC GOITER *

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The major interest in the pathology of the exophthalmic goiter syndrome still hinges about the thyroid gland as it has done in an indefinite manner since 1840 and in a specific way since Gauthier and Möbius in 1886 and 1887 advanced the hypothesis that the symptom-complex was dependent on hyperactivity of the thyroid gland with a resultant thyroid intoxication.

But, notwithstanding the large amount of study and experiment that has been directed to this subject during the past twenty-five years, the thyroid hypothesis of the etiology of the exophthalmic goiter syndrome still remains an hypothesis.

In recent years the clinical evidence, especially that of surgical therapy, tends to support the thyroid hypothesis, while at the same time the evidence from pathologic anatomy, chemistry and pathologic physiology rather suggests that the thyroid does not play a primary rôle in the production of the symptom-complex.

## I. ANATOMIC CHANGES IN THE THYROID

From the standpoint of pathologic anatomy the changes are body-wide and highly variable. The most striking and most constant are those of the lymphoid and thyroid tissues.

Confining ourselves to the thyroid changes, widely different views have been expressed as to their significance. Thus Virchow,[1] summarizing the literature to 1863, stated that there "was neither a distinct variety

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* Read in the Symposium on Internal Secretions in the Sections on Practice of Medicine and Surgery of the American Medical Association, at the Sixty-Third Annual Session, held at Atlantic City, June, 1912.

[1] Virchow: Die krankhafte Geschwülste, 1863, iii, 74.

nor a distinct size nor yet a distinct stage of goiter associated with the symptom-complex," and concluded that such changes could not be considered as causal or primary.

Beginning particularly with the development of the thyroid hypothesis of Gauthier and Möbius and the operative treatment of exophthalmic goiter, the thyroid changes were more extensively studied and the trend of opinion favored the view that the morphologic changes were constant and characteristic. Thus the published studies of Greenfield,[2] Haemig,[3] Farner,[4] Lewis,[5] Ewing,[6] Wilson[7] and many others strongly supported such a view.

On the other hand, the studies of Virchow,[1] A. Kocher,[8] Reinbach,[9] Brissaud,[10] MacCallum,[11] and Simmonds[12] have shown a wide range of morphologic changes in the thyroid.

My own experience during the last seven years includes careful anatomic examinations of 137 operative and autopsy specimens of the thyroid from patients with exophthalmic goiter. The results of part of this series have already been published,[13] and those still unpublished will not materially alter the proportion or types of changes then noted. I found that the symptom-complex as at present diagnosed was not associated with either constant or characteristic changes in the thyroid, although in about three-fifths of all cases some degree of active hyperplasia was present at the time of removal.

Thus, as larger series of cases have become available for study, opinion is again returning to the view of Virchow, that the symptom-complex may be present with the thyroid in any anatomic state—normal, actively hyperplastic, colloid, in glands the seat of tumors, benign or malignant, or with high degrees of atrophy. These changes, as has long been known, are those common to

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2. Greenfield: Brit. Med. Jour., 1897, ii, 1261.
3. Haemig: Arch. f. klin. Chir., 1897, lv, 1.
4. Farner: Virchows Arch. f. path. Anat., 1896, cxliii, 509.
5. Lewis: Surg., Gynec. and Obst., 1906, iii, 477.
6. Ewing: Tr. Assn. Am. Phys., 1906, xxi, 567.
7. Wilson: Am. Jour. Med. Sc., 1908, cxxxvi, 851.
8. Kocher, A.: Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1902, ix, 1; Arch. f. klin. Chir., 1910, xcii, 442; Virchow's Arch. f. path. Anat., 1912, ccviii, 86.
9. Reinbach: Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1901, viii, 247.
10. Brissaud: Semaine méd., 1895, xv, 326.
11. MacCallum: THE JOURNAL A. M. A., Oct. 5, 1907, p. 1158.
12. Simmonds: Deutsch. med. Wchnschr., 1911, xxxvii, 2164.
13. Marine and Lenhart: Arch. Int. Med., 1911, viii, 265.

goiter in general, irrespective of their clinical association but with this difference that the proportion of glands showing active hyperplasia (developing goiter) at the time of operation is at present higher in a series of exophthalmic goiters than in ordinary goiter.

## II. IODIN CONTENT OF THE THYROID

Turning to the iodin contents of thyroids in relation to goiter formation, it is now established that the iodin content varies with the amount of visible colloid and inversely with the degree of active epithelial hyperplasia. This relation has been found constant for all mammalian thyroids thus far studied.

The effect of iodin on active thyroid hyperplasia has also been studied in some detail and in all the so-called simple hyperplasias of man and the lower animals it has been found that following the administration of iodin there is a rapid storage of iodin in the thyroid in association with the accumulation of colloid and a progressive involution (throughout three to five weeks) of the active hyperplasia to its colloid or resting stage.

With these facts concerning the iodin relations established for simple thyroid hyperplasia (goiter) and with the anatomic similarity of the hyperplasia found in exophthalmic goiter with that occurring spontaneously in simple goiter or in experimentally induced hyperplasia, it is of importance to ascertain whether iodin induces the same effects on the hyperplasia of exophthalmic goiter. Clinically both good and bad results have followed the use of iodin in exophthalmic goiter and it is still the prevailing opinion that iodin should not be used in this disease, although clinicians generally have noticed a reduction in the size of the thyroid following its use. While individual cases, in which the histologic structure and the iodin content of the gland have been examined following the administration of iodin, have from time to time been reported by Oswald[14] and others, it has only been during the last two or three years that this question has received extended investigation. Thus A. Kocher[15] reported a series of 160 thyroid examinations and in those known to have received iodin he found that there had been a definite storage of iodin in the thyroid and that this accumulation of iodin was associated with an involution of the active hyperplasia. It was on the basis of this finding that Theodore Kocher introduced the hypothesis of active and inactive iodin in the thyroid as an attempt to explain the well-known clinical observation that the highest iodin contents were usually found in the milder cases.

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[14] Oswald: Virchows Arch. f. path. Anat., 1902, clxix, 453.
[15] Kocher, A.: Arch. f. klin. Chir., 1910, xcii, 442.

Last year Lenhart and I[13] reported our observations on the effect of feeding iodin, on the iodin content and histologic structure in a series of fifteen cases of undoubted exophthalmic goiter. Our findings were similar to those of Kocher, that a rapid storage of iodin in the gland takes place and an involution of the hyperplasia to the colloid state results.

Thus the thyroid hyperplasia of exophthalmic goiter behaves toward iodin exactly as does any other thyroid hyperplasia of any animal thus far investigated and one can with certainty conclude that physiologically as well as anatomically there is as yet no known attribute of the thyroid of exophthalmic goiter that may not be shared by the thyroid overgrowth of any other clinical association.

## III. EFFECT OF THE ADMINISTRATION OF THYROID

Another series of experiments that have been the obvious though natural outcome of the Gauthier-Möbius hypothesis of the thyroid origin of exophthalmic goiter are those in which the effects of extracts of thyroid, or desiccated thyroid from exophthalmic goiter, on normal animals, on cretin or myxedematous animals, and lastly on exophthalmic goiter patients themselves have been studied.

To review briefly the development of this phase of the subject: It had been observed from the beginning of thyroid opotherapy that patients with exophthalmic goiter were relatively more affected than were patients with ordinary goiter, and clinically this reaction was an exaggeration of the existing symptom-complex. Ballet and Enriquez[16] had reported the experimental reproduction of the exophthalmic goiter symptom-complex in dogs by the use of large amounts of desiccated thyroid, and Notthaft[17] had observed the development of the symptom-complex in a normal individual following the use of excessive amounts of desiccated thyroid. All these observations were interpreted as proof of the Gauthier-Möbius hypothesis.

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[16] Ballet and Enriquez: Semaine méd, 1894, xiv, 66.
[17] Notthaft: Centralbl. f. inn. Med., 1898, xix, 353.

On the other hand; the more extensive experimental observations which followed this new suggestion have not confirmed the earlier views. Thus Cunningham[18] failed to produce in birds or mammals any symptom-complex resembling exophthalmic goiter. In our feeding experiments on dogs we were unable to produce any noteworthy rise in the temperature or pulse-rate or to bring about thyroid enlargement or exophthalmos by feeding thyroid either fresh or dry. The effect of thyroid substance was in general proportional to the iodin content, and Stoland[19] has recently reported similar results from the use of normal and hyperplastic dog thyroids on rats and guinea-pigs.

The latest extensive work is that reported by Carlson, Rooks and McKie,[20] who likewise have been unable to reproduce any symptom-complex resembling exophthalmic goiter. All the recorded observations, whether interpreted positively or negatively, have shown essentially identical pharmacologic reactions for the thyroid substance—increased appetite, loss of body weight, diarrhea, increase in the nitrogen and carbon dioxid output and oxygen consumption—and the different conclusions reached seem to be dependent on the interpretations placed on this common pharmacologic reaction.

Since iodin has been found to have such an intimate relation to the physiologic activity of the thyroid, and since large doses of iodin have long been known to exaggerate the symptom-complex in certain types of exophthalmic goiter, this element also has been advanced as an etiologic factor in exophthalmic goiter, and Kocher has introduced the term "iodin-Basedow" in this connection. Kocher,[21] Goldflam,[22] Cerioli[23] and others have reported cases in which an existing symptom-complex was exaggerated and also cases in which the symptom-complex was initiated by the excessive use of iodin. But here, as in the thyroid feeding experiments, extensive experimental investigation has revealed a common pharmacologic and toxicologic action for iodin in all forms of thyroid hyperplasia and the different conclusions are dependent on the different interpretations that have been placed on this common action.

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[18] Cunningham: Jour. Exper. Med., 1898, iii, 147.
[19] Stoland: Am. Jour. Physiol., 1912, xxx, 37.
[20] Carlson, Rooks and McKie: Am. Jour. Physiol., 1912, xxx, 129.
[21] Kocher: Arch. f. klin. Chir., 1910, xcii, 1166.
[22] Goldflam: Berl. klin. Wchnschr., 1911, xlviii, 423.
[23] Cerioli: Policlinico, Rome, 1909, xvi, sez. prat., 527.

Turning to the experiments with exophthalmic goiter thyroid, Soupault[24] found that feeding such gland to guinea-pigs produced less evidence of physiologic activity than did normal human or sheep thyroid. Hutchison[25] also fed the thyroid proteins of exophthalmic goiter glands to patients and later used extracts experimentally on dogs with negative results. Gley and Cleret[26] injected intravenously into dogs and rabbits serums obtained from six patients with exophthalmic goiter, but observed no changes in the blood-pressure or pulse-rate. Gley[27] used fresh extracts of thyroids from patients with exophthalmic goiter, simple goiter, thyroid cyst fluid and ox thyroids injected intravenously into dogs in doses of 0.5 gm. per kilogram. He found, as others had found, that all thyroid extracts tend to cause a slight fall of blood-pressure and was unable to make out any difference between the action of exophthalmic goiter extracts and those of ordinary goiter. The normal ox thyroid extracts induced about twice the effect on the circulation that like amounts of exophthalmic goiter extracts did. Schoenborn[28] had two years before used fresh normal salt solution-glycerin extracts of seven exophthalmic goiters, three ordinary goiters and twenty-three thyroid tumors. He used these extracts intravenously in cats and rabbits and came to the conclusion that there was no essential difference in the cardiovascular action of the thyroid juice dependent on clinical association, but that the slight differences noted were due to differences in the amount of iodized thyreoglobulin, as von Cyon and Oswald[29] had found.

Recently Klose[30] and Lampe, Liesegang and Klose[31] have repeated some of this work and have arrived at opposite conclusions. They urge as objections to all previous work that the exophthalmic goiter thyroid extracts must be fresh (from twenty to thirty minutes after operative removal from the patient) and that one must use susceptible animals which in their experience are to be found in nervous, irritable fox-terrier dogs. They were able to reproduce a pulse and temperature reaction lasting from two to six days in some ways similar to the postoperative pulse and temperature reaction in exophthalmic goiter. Their conclusion is the more striking when they state that the toxic principle is present only in the freshest gland and then, without any knowledge of the iodin content of the glands with which they worked, they believe the toxicity is dependent on a masked and abnormal iodin combination which they designate as "Basedow-iodin" because potassium iodid and sodium iodid introduced intravenously in large doses produced, in their hands, a somewhat similar temperature and pulse reaction and even the so-called Basedow blood-picture.[32]

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[24] Soupault: Rev. de Neurol., 1897, p. 630.

[25] Hutchison: Brit. Med. Jour., 1896, ii, 896.

[26] Gley and Cleret: Jour. de physiol. et de path. gén., 1911, xiii, 928.

[27] Gley: Jour. de physiol. et de path. gén., 1911, p. 955.

[28] Schoenborn: Arch. f. exper. Path. u. Pharmakol., 1909, lx, 390.

[29] Von Cyon and Oswald: Arch. f. d. ges. Physiol. (Pflüger's), 1901, lxxxiii, 199.

[30] Klose: Arch. f. klin. Chir., 1911, xcv, 649.

[31] Lampe, Liesegang and Klose: Beitr. z. klin. Chir., 1912, lxxvii, 601.

Baruch[33] has reported the production of exophthalmos, tachycardia, glycosuria and lymphocytosis in dogs, rabbits and rats by injecting whole-gland emulsions into the peritoneal cavity in from 5 to 20 c.c. doses. His results differ from those of Klose in that he was able to reproduce the clinical picture of exophthalmic goiter with extracts of several clinical types of goiter in young bitches irrespective of their nervous temperament.

Another series of observations bearing on the same phase were those reported by Fonio[34] from Kocher's clinic. Fonio studied the effects of exophthalmic goiter thyroid preparations on the nitrogen metabolism and the blood-picture of myxedema patients with the object of finding out whether exophthalmic goiter thyroids were therapeutically more active than ordinary thyroid. He concludes that exophthalmic goiter thyroid is no more potent than other thyroid preparations of like iodin content or, in other words, that the activity varies with the iodin content.

During the past year, in Dr. Hoover's clinic at the Lakeside Hospital, we have made some observations bearing on this same question, but from another angle. We have fed desiccated exophthalmic goiter thyroid to patients with the complete exophthalmic goiter syndrome, in from 0.5 to 1.0 gm. doses daily for from ten to fifteen days, both with and without the use of iodin in addition. These thyroid preparations were low in iodin, varying from 0.01 to 0.03 per cent. In two patients the gradual fall in the pulse-rate due to rest continued throughout the experiment, while a third patient, who was getting 1 gm. of desiccated exophthalmic goiter thyroid daily and 0.7 c.c. syrup of ferrous iodid three times daily, showed no appreciable difference in the average pulse-rate at the beginning and at the end of the eleven days' feeding. We have also used normal desiccated sheep's thyroid in about one-third the above doses in typical cases of exophthalmic goiter observed at the Lakeside Hospital Dispensary and in such cases it is well known that one usually gets in the course of a week's feeding a definite increase in the pulse-rate if there has been a definite increase in the total metabolism with a loss of body weight. One may therefore conclude that the effect of exophthalmic goiter thyroid when fed to patients with the exophthalmic goiter syndrome varies with the iodin content, and in this respect is similar to like preparations of other clinical associations.

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[32] Bardenhewer (Arch. f. klin. Chir., 1912, xcvii, 729) has repeated Klose's experiments with potassium iodid and sodium iodid, and in six dogs he was unable to obtain any reaction comparable to those reported by Klose. That is, he found no noteworthy change in the pulse-rate or temperature, no albuminuria or glycosuria, no tremor or sweating or exophthalmos as reported by Klose. Klose in a short reply to this work (ibid., p. 829) states that these experiments do not repeat his because Bardenhewer did not use the nervous, excitable fox-terriers which Klose claims are absolutely essential.

[33] Baruch: Zentralbl. f. Chir., 1912, xxxix, 316; 1911, No. 35.

[34] Fonio: Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1911, xxiv, 123.

## SUMMARY

1. Neither specific nor constant anatomic changes in the thyroid of exophthalmic goiter have as yet been demonstrated.

2. The iodin content, the storage of iodin in the gland and the involution of active hyperplasia by the use of iodin are, so far as is at present known, identical with those iodin relations common to other clinical associations.

3. The thyroid of exophthalmic goiter has no different pharmacologic action on animals or therapeutic action on myxedema or toxic action on patients with exophthalmic goiter than thyroid preparations of other clinical associations with like iodin contents.

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# THE ALLEGED EXISTENCE OF ADRENALIN (EPINEPHRIN) IN PATHOLOGICAL SERA.*

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PLATES 77–80.

In a previous paper,[1] I concluded that no proof had hitherto been given that adrenalin is ever present in detectable amount in normal sera from the general circulation. I have since examined by similar methods a number of sera from clinical cases. In the first paper, the literature on the alleged detection of adrenalin in pathological sera and the so-called estimation of the variations in the adrenalin content of the blood in different diseases was sufficiently covered, and few further references will be given here.

In describing my own results, I shall examine and illustrate by special experiments the difficulties in the methods hitherto employed which, in my opinion, render the search for adrenalin under any conditions in the general circulation quite unpromising and at present devoid of practical importance in clinical work. As will be shown, the methods used do suffice for the detection of adrenalin in serum obtained more or less directly from the adrenal glands when they are excited to activity. Accordingly, the failure of the methods in the case of the general mixed blood is due, not to their inadequacy to detect adrenalin, but to the absence of that substance in the general circulation or to its presence in a concentration below the limit recognizable by the so-called biological tests.

Since these tests, with the objects employed, depend solely on the interpretation of tracings, it is necessary to reproduce a considerable number of these and to refer to them in detail.

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* Received for publication, March 18, 1912.

[1] G. N. Stewart, *Jour. Exper. Med.*, 1911, xiv, 377.

## TECHNIQUE.

The technique was the same as that described in the previous paper, segments of rabbit small intestine and of rabbit uterus constituting the test objects. The animals were killed by a blow. In a few experiments the rabbit was kept under urethane given by stomach tube. But this seemed to cause irregularity in the beats of the intestinal segments, rendering them somewhat unsatisfactory till after repeated washing. The oxygen stream entered the apparatus by a side tube opening low in a small cylinder. The tube was of capillary bore so as to give small bubbles of oxygen, and at the same time to take up only a little of the liquid in the cylinder.

It is scarcely necessary to mention that the steadiness and sufficiency of the oxygen stream were sedulously controlled. Otherwise grave errors of interpretation of the changes in the curves can very easily occur. For example, in figure 1 an intestine preparation was beating in Ringer. At 6 the oxygen supply was increased and a great increase in the size of the beats occurred, both the maximum contraction and the maximum relaxation being markedly augmented. At 6 in figure 31 precisely the opposite effect at first accompanied the increase in oxygen; viz., a great drop in tone, succeeded by a more lasting and steady rise.

The condition of the preparation at the moment is, without doubt, an important factor in relation to the effect of variations in the oxygen supply. In figure 8, with a uterus preparation which was contracting rather feebly in Ringer, an increase in the oxygen at 6 caused a prompt and great increase both in the individual beats and in tone, which, if it had coincided with the addition of a suspected serum, might easily have been taken for an adrenalin effect.

The liquid in the cylinder was always replaced by running the new liquid into the bottom of the cylinder and allowing the displaced liquid to overflow into the bath. A separate narrow tube piercing the rubber cork which closed the bottom of the cylinder and connected with a small funnel was first employed for this purpose, but it was soon discarded as presenting no advantages, and in the great majority of the experiments the new liquid was added by passing a pipette drawn to a fine point down to the bottom of the cylinder. The pipette was slipped down along the glass and did not interfere at all with the preparation. The pipette was bent at a right angle so that when the point was in the cylinder its other limb, connected with a small rubber tube and glass mouthpiece, was horizontal. The horizontal limb was graduated so that the amount of liquid introduced was accurately known. As a rule, it was just enough to fill the cylinder, and displaced, therefore, all the liquid previously present. Thus the preparation was always completely immersed in liquid and the disturbance caused by alternately emptying and filling the cylinder, during which the preparation alternately sinks to the bottom and is floated up again to the top, being now in contact with gaseous oxygen and now with liquid, was avoided.

The apparatus is conveniently made in different sizes by cutting down glass T-pieces. Half a dozen cylinders of different capacity were constructed but two were used mainly: one, referred to as the larger apparatus, holding without the preparation 4.5 c.c.; the other, referred to as the smaller apparatus, holding without the preparation 2.5 c.c. When the supply of serum was abundant, the former was used; when it was scanty, the latter. When the preparation was in position, 4 c.c. of serum or other liquid sufficed completely to displace the contents of the larger apparatus, and 2 c.c. to displace the contents of the smaller.

The apparatus used and the weight of the preparation after drying with blotting paper were always noted since the action of a substance such as adrenalin is influenced, not only by its concentration, but also by the relation its total amount bears to the mass of the tissue affected.

What has just been said of adrenalin is true also of serum. The necessity of controlling this factor is well illustrated in figure 2. At 33, while an intestine preparation was contracting rather feebly in Ringer, 1 c.c. of dog serum was introduced into the small apparatus. It displaced about half of the Ringer and was soon mixed up with the rest by the bubbles of oxygen forming a serum diluted with its own volume of Ringer's solution. The size of the beats was much increased with very little increase of tone. At 34, 2 c.c. of the serum was run in, displacing all the diluted serum, and a marked increase of tone occurred.

The preparation was always tested with adrenalin solutions of different concentrations, made up in the suspected serum, or in a control serum, or in Ringer's solution, or in all three. Such comparisons for each preparation are essential.

The adrenalin chlorid solution (1 : 1,000) of Parke, Davis and Company was employed. As a rule, no bottle was used more than three or four days after it had first been opened. For the more important experiments a fresh bottle was invariably opened. The adrenalin was of course kept in the ice chest. After the application of a given serum or solution the preparation was thoroughly washed with Ringer's solution (introduced by the pipette already mentioned) before a new observation was begun. Unless otherwise stated, the liquid tested was introduced while the preparation was beating in Ringer.

## COMPLICATING EFFECT OF SERUM ON THE TESTS.

The difficulty of detecting adrenalin in blood serum is greatly enhanced by the fact that serum itself exerts a marked influence on the biological objects which are probably the most sensitive to adrenalin; namely, the intestine and uterus. Serum causes a great increase of tone in both objects. In the case of the intestine, the adrenalin inhibition must overcome the pressor[2] effect of the serum before it can manifest itself. In the case of the uterus, at least in that of the adult uterus which has been once impregnated, the serum and adrenalin effects are in the same direction, both causing an increase of tone. In this case, therefore, the presence of adrenalin in a given serum can be revealed only by an increase in the effect of the serum. The tone-increasing power of foreign serum (horse)

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[2] Although the term "pressor" is usually employed by physiologists in relation to increase in tone of blood-vessels, it will be convenient sometimes to use it here to denote an increase in tone of smooth muscle of the intestine and uterus, and no ambiguity can arise from this.

on the smooth muscle of perfused blood-vessels of cats, recently investigated by Schultz,[3] opens to objection the perfusion test of Trendelenberg. Such test objects are, therefore, very much less sensitive for adrenalin when it is in serum or blood than when it is in simple solutions, like Ringer's. This lack of sensitiveness in serum will continue to be a difficulty until means have been adopted either to eliminate the pressor effect of the serum without at the same time destroying the adrenalin or to eliminate the adrenalin without affecting the pressor effect of the serum. The observation of Schultz[4] that, after boiling, the pressor action of serum on segments of intestine is much diminished, is of interest in this connection.

Each specimen of serum, not only from different animals or different normal human beings or different clinical cases, but from different points in the circulation of one and the same animal, or from the same point at different times, might be expected to differ in its pressor activity on the organs with smooth muscle. As a matter of fact, this has, in a large number of instances, been shown to be the case. It is clear that if an intestine preparation is beating in serum A and the replacement of serum A by serum B causes a diminution in tone, it cannot be concluded that this is due to adrenalin or to an adrenalin-like substance, unless it is known that the pressor effect of B (apart from any adrenalin content it may have) is not less than that of A. If it is less than that of A, a fall of the curve may be expected to occur when B displaces A, even if B is altogether free from adrenalin. In the present as in the previous investigation, this difficulty is eliminated as far as possible by using a second smooth muscle preparation (uterus) on which the normal action of adrenalin is to produce an increase of tone. If the fall of tone supposed to have been caused by B in the intestine preparation when it displaced A is due to the pressor effect of B on the smooth muscle being less than that of A, then serum B ought also to cause a fall of tone in a uterus preparation previously beating in serum A. On the other hand, if the action is due to adrenalin or to a sympatho-mimetic substance, B ought to increase still more the tone of the uterus preparation already increased by A. Probably the same would apply to the smooth muscle of blood vessels, which might be substituted for the uterus in discriminating between changes of tone produced by adrenalin and those produced by differences in the pressor activity of different sera for smooth muscle. The complicating effect of serum on the detection of adrenalin added directly to it was illustrated by special experiments in which solutions of adrenalin of different strengths in fresh calf serum were compared with solutions of equal strength in Ringer's.

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[3] W. H. Schultz, *Jour. Pharmacol. and Exper. Therap.*, 1912, iii, 299.

[4] W. H. Schultz, *Jour. Pharmacol. and Exper. Therap.*, 1909–10, i, 549.

In figure 3 it is shown that with the volumes of liquid employed (enough to displace the liquid in the larger apparatus) a solution of adrenalin in serum (1 : 10,000,000) produced a distinct preliminary inhibition before the increase of tone due to the serum (observation 31), although the diminution of tone was much less than in observation 33 in the same figure, where, after washing the preparation with Ringer's solution, the latter was replaced by adrenalin (1 : 10,000,000) in Ringer. With the adrenalin-Ringer solution the period of diminished tone was succeeded by a period of increased tone with some increase in the force of the beats, although the maximum tone reached was far less than with the adrenalin-serum. In figure 4 is shown a much more marked preliminary inhibition of the same intestine preparation by adrenalin (1 : 5,000,000) in the calf serum. In figure 5, where a much weaker solution of adrenalin (1 : 25,000,000) in Ringer was made to replace Ringer (observation 20), the inhibitory effect is unmistakable. On the other hand, at 23 and 27, adrenalin (1 : 25,000,000) in the serum produced little if any preliminary inhibition of the intestine, although the effect of the adrenalin might possibly be recognized and to a certain extent estimated by comparing the amount and especially the duration of the increase in tone in the serum-adrenalin curves with the same changes in the serum curve (observation 25). But the fact that even with the conditions kept as constant as possible the increase of tone was much more transient in 23 than in 27, although in both cases it was distinctly less persistent than in 25, where the serum alone was added, shows how difficult it would be to detect adrenalin in serum with certainty in anything like this dilution, even where the control serum is identical with that containing the adrenalin, a condition of course never realizable in testing serum for clinical purposes.

In figure 6, with the same intestine preparation, adrenalin in serum (1 : 50,000,000, observation 35) produced an effect so little different from that of the serum itself (37) that its detection could not be relied on. The somewhat quicker descent after the maximum increase of tone in observation 35 was too little marked to be used as a criterion. With a solution of adrenalin in Ringer of the strength of 1 : 50,000,000 added to the same preparation after washing with Ringer, the typical adrenalin reaction was perfectly distinct (observation 39, figure 6). In these observations, then, where the adrenalin was actually added to the serum, and where, accordingly, the conditions for faultless control experiments existed, the test would unquestionably have failed for dilutions over 1 : 25,000,000, and probably would have been too uncertain for considerably stronger solutions, and this, too, with a sensitive intestinal preparation.

With a preparation which was less sensitive, although from the same rabbit, and not so long isolated (figure 7), a 1 : 5,000,000 dilution of adrenalin in serum was required (observation 7) to give an unequivocal effect as compared with the serum itself (observation 4). At 11 the adrenalin-serum was again added. In both cases although there was a slight initial inhibition, the main effect was a more prompt relaxation after the preliminary rise due to the serum. The maximum increase of tone, however, was practically as great with the serum containing adrenalin as with the normal serum.

In figures 8 to 12 the same serum and solutions of adrenalin in serum were tested on a rabbit uterus preparation. At 5 (figure 8), Ringer was replaced by adrenalin (1 : 25,000,000) in serum, and at 7 by adrenalin (1 : 25,000,000) in Ringer. In figure 9 at 10 and 12, solutions of adrenalin (1 : 50,000,000) in serum and Ringer were similarly compared; in figure 10, solutions of 1 : 10,000,000; in figure 11, solutions of 1 : 5,000,000. In all of these dilutions the presence of serum notably modified the curve, causing a greater increase of tone and a longer and more complete cessation of contractions at the maximum of the tone increase. It is only with quite strong adrenalin solutions that the curve became essentially the same whether serum was present or not. Thus in figure 12, adrenalin in serum (1 : 50,000, observation 29) produced practically the same effect as adrenalin in Ringer (1 : 50,000, observation 34); *i. e.*, a marked and durable increase of tone and stoppage of contractions.

### DETECTION OF ADRENALIN IN BLOOD FROM THE ADRENALS.

We shall now show that the method in our hands is capable of detecting adrenalin in serum when the blood, either undiluted or not greatly diluted with other blood, is obtained from the adrenal gland under conditions favorable to the liberation of adrenalin. This seems all the more necessary as the supposed proof of the liberation of adrenalin as a normal event rests mainly on indirect experiments.

If the question is to be properly tested it is clear that mechanical interference with the gland or with its blood supply must be avoided,[5] for it is more than probable that in asphyxia of the gland or when it is mechanically manipulated a certain amount of adrenalin may get into the circulation. This was tested as follows:

*Experiment of February 13, 1912.*—A dog weighing 7.5 kilos was anesthetized with ether. A cannula was inserted into the peripheral end of the right femoral vein and a sample of defibrinated blood obtained from it. The serum separated from this blood by the centrifuge is serum I. Then a cannula was inserted into the peripheral end of the transverse lumbar vein which courses over the left adrenal gland between the gland and the inferior vena cava. Care was taken as far as possible to avoid manipulation of the gland. The blood was allowed to escape from the vein while the cannula was being put in and the flow was never interrupted. Then the lumbar vein was ligated before it reached the gland, so that only the adrenal contribution passed through the cannula. A sample of blood was collected from the cannula, which yielded serum II. A third specimen of blood from which serum III was separated, was then taken at once from the adrenal cannula during vigorous massage of the gland with the fingers. Afterwards the rest of the blood in the animal was drawn from the right carotid and the separated serum labelled serum IV. All samples were at once put on ice and the centrifugalizing was conducted in a cool room. The two adrenals together weighed 1.3 grams. The tests with rabbit intestine and uterus (in the small apparatus) were begun within three hours of etherization.

In figure 13, observations 2, 4, and 9 show the increase of tone in the intestine preparation when Ringer's solution was replaced respectively by serum I, serum II, and serum III. Although the maximum increase produced by serum II is somewhat less than the maximum for the other two sera, this as already pointed out is in itself no evidence of the presence of adrenalin. The effect upon the same intestine preparation of replacing Ringer by serum III (as shown at 6 in figure 14) was very different. There was practically no increase of tone or only a very slight one, while the beats eventually became much smaller. This distinctly suggests the presence of adrenalin in considerable concentration. Observation 11 (figure 13) shows the effect of replacing Ringer by adrenalin 1 : 25,000,000 in serum IV. The maximum increase of tone was much less than that caused by serum IV itself, but there was no preliminary inhibition. The same was true of adrenalin 1 : 12,500,000 in serum IV (observation 13, figure 13), while adrenalin 1 : 12,500,000 in Ringer (observation 15) caused a distinct inhibitory effect. Adrenalin 1 : 1,250,000 in serum IV produced slight inhibition when it replaced Ringer (figure 15, observation 17), but this inhibition was succeeded by a very fair increase of tone, although the beats became much smaller. When Ringer was replaced by adrenalin 1 : 1,250,000 in Ringer at 19, figure 15, the inhibitory effect was quite pronounced. The tone of the preparation beating in Ringer (at 18) was about the same as before 17. In order to save space in grouping the tracings, the relative position of the curves to the base line was not preserved. The increase of tone produced by the adrenalin-serum was so much greater than that caused by serum III in figure 14 as to suggest strongly that if the absence of the pressor effect in serum III was due to its neutralization by adrenalin, the concentration of adrenalin in serum III must have been considerably greater than 1 : 1,250,000. This estimate was confirmed in figure 16 where the suspected serum was caused to replace a control serum. At 28, serum IV replaced Ringer, and at 29 serum IV was itself replaced by serum III. There was an immediate and considerable drop in the curve. At 36 in figure 16, Ringer was replaced by serum IV, and this at 37 by adrenalin 1 : 12,500,000 in serum IV. The inhibitory effect of the adrenalin-serum was so slight and transient that if the effect at 29 was due to adrenalin in serum III the concentration must certainly have been very much greater than 1 : 12,500,000. At 40, Ringer's solution was replaced by adrenalin 1 : 500,000 in serum IV. The fall in the curve was not quite as abrupt and certainly no more marked than at 29. The preliminary rise was exaggerated by accidental interference with the lever during the introduction of the adrenalin-serum. We can conclude with considerable probability that serum III contained not less than 1 : 500,000 of adrenalin.

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[5] Kahn, *Arch. f. d. ges. Physiol.*, 1911, cxl, 209.

By means of the uterus, confirmation was obtained for the conclusion that serum III contained a considerable amount of adrenalin and that the fall of tone produced by it when it displaced serum IV in contact with the intestine was not due to a much inferior tone-increasing power for smooth muscular structures in general. In other words, when, with a uterus preparation, serum III replaced serum IV the tone was still further increased instead of diminished. Four days afterward, the sera having in the meantime been kept in the ice chest, serum III caused only a slight and transient rise when it replaced serum II (figure 24, at 10). Serum II replaced Ringer at 9 in the same figure and produced a good increase of tone.

We may consider it as proved, then, that with an adrenal gland which was not giving off adrenalin to the blood stream in detectable amount, vigorous massage caused the passage into the venous blood coming from the gland of sufficient adrenalin to give a concentration of not less than 1 : 500,000. In a previous experiment in which the cannula was inserted in the same way, but no particular care was taken to avoid disturbance of the gland, both intestine and uterus tests showed adrenalin in blood collected from the cannula. The avoidance of practically all disturbance of the gland was realized in the following experiment.

*Experiment of February 14, 1912.*[6]—A dog weighing 10.2 kilos was etherized at 8:55 A. M., and carotid and tracheal cannulæ were inserted. The carotid cannula was connected with a manometer. Electrodes were placed on the right and left great splanchnic nerves just central to the adrenals. Ligatures were placed in position around the inferior vena cava; (a) just above the entrance of the adrenal veins, (b) below the entrance of the renal veins. A ligature was also placed in position around the right renal vein and a cannula was inserted through the left renal vein into the inferior vena cava. The ligatures were left quite loose. At 9:37 A. M. sample I (47 c.c. of blood) was taken from the vena cava through the cannula before any of the ligatures had been tightened. At 9:40 sample II (29 c.c.) was taken from the vena cava after tightening all three ligatures so as to make a closed sac of the cava, except for blood from the adrenals and the lumbar veins crossing the glands. The lumbar veins were not tied in order to avoid interference with the glands, and lest the adrenal blood should fill the pocket and cannula so slowly that there would be danger of clotting. At 9:45 A.M. sample III (50 c.c.) was collected from the cannula in the cava under the same conditions as sample II, except that both splanchnics were stimulated continuously during collection. Before simultaneous stimulation, the nerves were excited separately to test the effect on the blood pressure. The left was more active than the right, although each produced a decided rise. They were stimulated together for six minutes during which time the blood was collected. The venous pocket filled but slowly. After sample III was collected the nerves were again separately tested with regard to blood pressure and were found active. At 9:53 sample IV, consisting of as much blood as could be obtained from the carotid artery, was collected at the end of the experiment. The sera were separated at once by the centrifuge. Samples I and IV were free from hemoglobin; II and III were slightly and equally tinged. All were kept in the cold, and the tests with intestine and uterus from a pregnant rabbit weighing 1,770 grams were begun four hours after the blood was drawn.

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[6] This protocol, as well as those of the experiments of February 16, January 19, and January 22, is extracted from the notes of Dr. J. J. R. Macleod, who was good enough to furnish me with the various specimens of blood which I afterwards tested, a very material service in the investigation.

In figure 17 at 20, Ringer, in which an intestine preparation was beating, was replaced by serum IV. The usual increase of tone occurred, and when the tracing had become horizontal serum IV was replaced by serum III at 21. An immediate and sharp drop in the curve took place, indicating the possibility that adrenalin was present. At 22, when the tone had recovered somewhat and the curve was again horizontal, serum III was in turn displaced by serum IV and the curve rose again. After washing, the Ringer was replaced at 24 by serum IV, and this at 25 by serum II. Instead of a drop there was no change, or perhaps a slight rise. At 28 Ringer was replaced by serum IV, and this at 29 by adrenalin 1 : 1,000,000 in serum IV. The same prompt drop occurred as in 21. In proportion to the height of the curve at the time the adrenalin serum was added, the drop was fully as great as with serum III and the subsequent recovery of tone was somewhat slower. If, then, the effect in observation 21 was due to adrenalin, it may be concluded that the concentration of adrenalin in serum III was a little but not much less than 1 : 1,000,000. Of course, when adrenalin in Ringer's solution in this concentration (1 : 1,000,000) was added (at 40), with the preparation already beating in Ringer, the inhibitory effect was much more pronounced. The preparation weighed 0.41 of a gram.

Results with a uterus preparation (weighing 0.65 gm.) from the same rabbit are given in figure 18. At 42 Ringer was replaced by serum IV, and this at 43 by serum III, which caused a further increase of tone with diminution or temporary cessation of the contractions at the maximum of the increase in tone. It may accordingly be inferred that the diminution of tone in the intestine caused by serum III when it replaced serum IV was not due to the inferiority of serum III as compared with serum IV in tone-increasing (pressor) power for smooth muscular structures. It must, then, have been due to some substance in it which increased the tone of the uterus while diminishing that of the intestine; *i. e.,* a substance like adrenalin. At 46 (figure 18) serum IV replaced Ringer, and was itself replaced at 47 by serum II. There was no increase of tone, but possibly some fall. At 50 Ringer was replaced by serum IV, and this at 51 by adrenalin 1 : 1,000,000 in serum IV. A prompt increase of tone ensued. It should be noted that the adrenalin-serum was introduced at the bottom of a contraction and the first portion of the rise, probably to the small nick in the curve, would almost certainly have occurred in any case. At 43 serum III was introduced at the top of a contraction. The increase in tone produced by the adrenalin-serum is, therefore, not very much greater than that caused by serum from the vena cava pocket collected during stimulation of the splanchnics. This strengthens the inference from the observations on the intestine that the adrenalin content of serum III was probably somewhat, but not much, less than 1 : 1,000,000.

Observations 53 and 54 (figure 18) are an almost exact repetition of observations 42 and 43 on the same uterus preparation after it had been well washed with Ringer and show that the rise of tone produced by serum III in the first observation was not accidental.

If adrenalin is really present in a serum it is to be expected that on keeping it in this alkaline medium even at a low temperature it will, after a certain time, disappear. Sera III and IV of February 14 were, therefore, again tested after they had stood in the ice chest for a time. The results on the intestine are shown in figure 19. At 52 Ringer was replaced by serum IV and this at 53 by serum III. At 55 Ringer was replaced by serum IV, and this at 56 by adrenalin 1 : 5,000,000 in serum IV. The tone was slightly increased by this adrenalin solution, but a stronger solution of adrenalin (1 : 500,000) in serum IV, when it was made to replace the weaker solution without washing the preparation, caused a fall (observation 57) much greater and more permanent than that caused by serum III. At 59 Ringer was replaced by serum IV, and this at 60 by adrenalin

1 : 1,000,000 in serum IV. The fall of tone is here also much greater and more lasting than that induced by serum III in observation 53, showing that now the latter contains much less than 1 : 1,000,000 adrenalin, whereas forty-eight hours before, judged by the same criteria, it contained but little less than 1 : 1,000,000. Observation 56 indicates that its content is still much more than 1 : 5,000,000. We can conclude that a great part of the adrenalin, but not the whole, has disappeared from the serum in forty-eight hours.

In passing, it may be noted that the absolute amount of the effect produced by serum III at 53 was little if at all inferior to that produced in figure 17 (observation 21). But in figure 19 the sensitiveness of the preparation to adrenalin was much greater. This shows how futile comparisons on different intestine preparations may be.

That some adrenalin was still present in serum III after three days in the ice chest was indicated by observation 29 (figure 20) on a uterus preparation from an animal probably fully grown but not pregnant. At 28 Ringer was replaced by serum I of February 14; the tone was increased and also the size of the contractions. At 29 this serum was replaced by serum III of February 14. A further increase of tone occurred which was of the same order of magnitude as that caused by a 1 : 5,000,000 solution of adrenalin in an "indifferent" serum (observation 26). At 25 the indifferent serum (serum V of February 16, see protocol, page 560) replaced the Ringer in which the preparation was initially beating. As far as can be judged from an experiment like this on the uterus preparation alone, we can perhaps conclude, knowing that we are really dealing with a serum which originally contained adrenalin, that after three days the adrenalin content of serum III has been reduced from nearly 1 : 1,000,000, to not more than 1 : 5,000,000 at most. From observations on the intestine it may be recalled that after the serum had been kept forty-eight hours it was inferred that it still contained much more than 1 : 5,000,000, although much less than 1 : 1,000,000.

So far, then, we have shown that during stimulation of both splanchnics adrenalin is given off to the blood in sufficient amount to cause a concentration of something like 1 : 1,000,000 in the blood obtained from the vena cava pocket. But of course not all this blood was from the adrenal. The adrenal contribution was probably diluted at least two or three times. The two adrenals together weighed 1.71 grams. In six minutes 50 c.c. of blood was collected from the pocket. If we suppose that the flow through the adrenals is as great as that attributed to the kidney,[7] 150 c.c. per 100 grams per minute;[8] and that stimulation of the splanchnics does not diminish it, this would only give 2.5 c.c. per minute for the adrenal contribution, or 15 c.c. for the six minutes. The concentration of adrenalin in the blood actually issuing from the glands in this experiment during stimulation of the splanchnics was, therefore, probably not less than 1 : 300,000. Even supposing that the concentration was two or three times greater, about 1 : 100,000, which must certainly be considered the extreme limit during what is in all probability a state of high functional activity of the glands, it may well be asked what chance there is of detecting adrenalin in the general blood stream in which the feeble trickle from the adrenal veins is diluted to an extreme degree. In a dog weighing 10 kilos probably not less than 2 kilos of blood passes through the heart in a minute,[9] and at least a third of this along the inferior cava from the portion of the body behind the orifices of the adrenal veins. In six minutes this would amount to 4 kilos of "indifferent" blood, into which would be poured during activity of the glands not more than 0.1 mg. of adrenalin giving, for the inferior cava blood, after receiving the adrenal contribution, an excess in adrenalin concentration of not over 1 : 40,000,000.

To determine whether during stimulation of the splanchnics adrenalin could be detected in blood removed from the inferior cava anterior to the adrenal veins, the following experiment was done.

*Experiment of February 16, 1912.*—A dog weighing 15 kilos was etherized at 9:55 A. M. Cannulæ were inserted into the trachea and carotid and a cannula into the right femoral artery. The abdomen having been opened, a catheter was passed through the central end of the right femoral vein and the end guided by the finger to a point just anterior to the entrance of the adrenal veins. The point of the catheter could also be seen through the walls of the veins. The catheter was as large as the femoral vein would admit. At 10:48, 45 c.c. of blood

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[7] Burton-Opitz, R., and Lucas, D. R., *Arch f. d. ges. Physiol.*, 1908, cxxiii, 553; Burton-Opitz, R., *idem*, 1909, cxxix, 208.

[8] K. O. Neumann (*Jour. Physiol.*, 1912, xliii, p. xxxi) gives a somewhat greater mean flow for the adrenals (2 to 3 c.c. per gm. per minute) in cats and rabbits.

[9] G. N. Stewart, *Jour. Physiol.*, 1897–98, xxii, 159.

(sample I) were collected from the right femoral artery. At 10:50 A. M., 40 c.c. of blood (sample II) were obtained from the catheter in the vena cava. Then during nine minutes from 10:51 to 11:00 A. M., 55 c.c. of blood (sample III) were collected from the catheter during stimulation of both splanchnics. In a preliminary test during which the carotid cannula was connected with a manometer and a blood pressure tracing recorded, it was shown that stimulation of each splanchnic caused a good rise in blood pressure. As the blood had partly clotted in the catheter the flow for sample III was slow. Stimulation was therefore discontinued, the catheter removed, washed free of clot, and reinserted. Then from 11:05 to 11:07½ A. M., 90 c.c. of blood (sample IV) were obtained from the catheter during stimulation of both splanchnics. Stimulation was then discontinued and from 11:11 to 11:14 A. M., 75 c.c. of blood (sample V) were drawn from the catheter. Finally, at 11:15 A. M., 220 c.c. of blood (sample VI) were obtained from the right femoral artery. All the samples were defibrinated and at once centrifuged in the cold. Sera I and VI were free from hemoglobin, serum II contained a slight trace, serum III more than II, although not deeply tinged. Sera IV and V were the same as II in this respect. The tests were begun less than four hours after the blood was obtained.

In figure 21 the Ringer in which an intestine preparation was beating was replaced at 65 by serum I, and this at 66 by serum III. For six or seven beats there was no change of tone or a slight increase, which was succeeded by a gradual fall. At 68 Ringer was replaced by serum I again, with the object of replacing this by serum III when serum I had been allowed to produce its effect on the preparation for a much longer time. At 69 some more of serum I was run into the cylinder as the oxygen stream had displaced some of the serum. A moderate rise of the curve took place bringing it up to the maximum first reached. At 70 serum I was replaced by serum III and a tardy fall ensued just as at 66. This effect is quite different from the adrenalin effect seen in figures 16 and 17. That it was not really due to adrenalin is indicated by observations 75 and 76 (figure 21). At 75 Ringer was replaced by serum II, and this at 76 by serum III, which produced not a fall but a further decided rise. Observations 72 and 73 are exact repetitions of 75 and 76, except that the serum added at 73 was serum IV of February 16. When tested against another presumably indifferent serum (*i. e.,* a serum free from detectable adrenalin), viz., serum VI of February 16, the two specimens of serum (III and IV) collected during splanchnic stimulation gave the same result, a further increase of tone of the intestine preparation (observations 49 and 50, and 81 and 82, figure 22). Earlier in the experiment it was shown with another intestinal preparation that when serum III or serum IV replaced serum I there was no diminution of tone. The fall in the curve succeeding the introduction of serum III at 66 and 70 therefore indicates not that this serum contained adrenalin, in detectable amount, but that its independent pressor effect on this preparation was less than that of serum I.

Figure 22 shows how marked and abrupt the changes in tone of an intestinal preparation may be when different sera, none of which can be shown to contain adrenalin, are caused to replace each other.

At 78 Ringer was replaced by serum VI, and this at 79 by serum V. The pressor effect of serum V was so much greater than that of serum VI that the curve was practically doubled in height. Had these two sera been added in the reverse order, serum VI must have produced a drop of the tone of the preparation already beating in serum V which might have stimulated an adrenalin effect. At 49 (figure 22) Ringer was replaced by serum VI, and this at 50 by serum IV. At 81 Ringer was replaced by serum VI, and this at 82 by serum III. In both cases the serum collected from the inferior vena cava during stimulation of the splanchnics produced a considerable additional increase of tone, although much less than that caused when VI was replaced at 79 by V.

The uterus likewise yielded no clear evidence of adrenalin in the blood of the inferior vena cava during stimulation of the splanchnics.

In figure 23 at 84 Ringer was replaced by serum II, which produced an increase in the tone and also in the rate of the contractions. At 85 serum II was replaced by serum III, which caused no further increase of tone, but, if anything, some inhibition, while adrenalin 1 : 1,000,000 in serum VI when it replaced serum VI at 93 occasioned a good rise. Serum VI, replacing Ringer at 92, caused the usual great increase of tone. In figure 24 at 2 Ringer was replaced by serum VI, and this at 3 by serum III, which certainly produced no further increase of tone. On the other hand, adrenalin (1 : 1,000,000 in serum VI) caused on replacing serum VI at 16 a very marked rise in the curve, the writing point going above the drum. The preparation was a fresh segment of a horn of the uterus used for observations 2 and 3 (figure 24), and was beating in Ringer when serum VI was added at 15. The replacement of serum V by serum III at 23, likewise caused no further change in the uterus curve which could be interpreted as indicating that serum III contained adrenalin. The replacement of Ringer at 22 by serum V increased the tone after an initial diminution.

Lest the increase of tone in the uterus occasioned by undiluted serum should have rendered it impossible for any clear further increase to occur when one of the control sera was replaced by a suspected serum, the observations illustrated in figures 23 and 24 were repeated with sera diluted with an equal volume of Ringer. In figure 25 an example of the results is given. Qualitatively they differ in no way from the others. At 5 Ringer was replaced by diluted serum VI, and this at 6 by diluted serum III, which in turn was replaced at 7 by diluted serum VI. Another experiment in which blood was collected in the same way from the cava (only the left splanchnic being stimulated) yielded similar negative results.

We conclude from all these observations that although the methods used clearly demonstrate the presence of a considerable concentration of adrenalin in blood coming from the veins of the adrenal glands during electrical stimulation of the splanchnics, they yield no unequivocal evidence of its presence in blood obtained from the inferior vena cava of dogs just anterior to the entrance of the adrenal veins during stimulation of these nerves. The cause of the negative result in this case is without doubt the high degree of dilution in the inferior cava of the blood coming from the adrenals.

Cannon's[10] positive result in cats under the influence of nicotine or emotion[11] might be explained by assuming (*a*) that these stimuli liberate more adrenalin than electrical stimulation of the splanchnic, or (*b*) that in a small animal a catheter produces a more effective block in the inferior cava, and thus allows the adrenal blood to be collected with less dilution.

Elliott's[12] failure to demonstrate exhaustion of adrenalin from the gland itself by electrical stimulation of the splanchnic may be due either to too short a period of stimulation or to too rapid exhaustion of the nervous path. The adrenalin discharged during six minutes of splanchnic stimulation in our experiment of February 14 was not more than 0.05 mg. for each gland. The glands weighed three to four times the average for Elliott's cats. With a six minute splanchnic stimulation probably little more than 0.01 mg. would disappear from a cat's adrenal. Elliott's adrenalin assay was accurate only to 0.01 mg. A cerebral puncture caused the disappearance of 0.3 mg. of adrenalin from one gland in four hours. Suppose that in four hours 150 kilos of blood passed through the cat's heart. If the liberation of adrenalin proceeded uniformly throughout the four hours and new formation is excluded, the liberated adrenalin could only maintain in the cava blood anterior to the adrenal veins an excess in concentration of 1 : 160,000,000. Even if the discharge of adrenalin by one gland is not augmented when the splanchnic supplying its fellow is cut, the liberation of 0.3 mg. from each gland in four hours would only bring up the excess to 1 : 80,000,000.

In two experiments venous blood from the adrenal gland was collected without admixture during stimulation of the corresponding splanchnic. For the reasons already given these experiments were not considered completely satisfactory. In both evidence of adrenalin was found in the blood from the adrenal.

*Experiment of January 22, 1912.*—Under ether anesthesia sample I was collected at the beginning of the experiment from a femoral vein of a dog weighing 10 kilos; sample II from the left adrenal vein before stimulation of the splanchnic; sample III from the left adrenal vein during stimulation of the left splanchnic. Sample IV, a mixture of blood from the femoral vein and carotid artery, was taken at the end of the experiment and after stimulation of the splanchnic was over; sample V consisted of a portion of the blood drawn from the carotid artery at the end of the experiment; and sample VI constituted the rest of the specimen of which sample V was a portion. Sample VI was evacuated as thoroughly as possible with the gas pump. As in the other experiments of this nature the activity of the splanchnic on the blood pressure was established by a preliminary test. In figures 26, 27, and 28 are seen specimens of the reaction of the intestine after the sera had stood for twenty-four hours in the ice chest. The smaller apparatus was used. The preparation weighed 0.39 gm.

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[10] Cannon, W. B., Aub, J. C., and Binger, C. A. L., *Jour. Pharmacol. and Exper. Therap.*, 1911–12, iii, 379.

[11] Cannon, W. B., and de la Paz, D., *Am. Jour. Physiol.*, 1911, xxviii, 64.

[12] Elliott, T. R., *Jour. Physiol.*, 1912, xliii, p. xxxii.

In figure 26 Ringer was replaced at 2 by serum VI and at 4 by serum V. Incidentally it is seen that the evacuation of the carbon dioxid did not affect the tone-increasing power of the serum. At 6 and at 8 Ringer was replaced by serum III, the preparation having been washed during the interval. The two curves are as nearly identical as can be expected, even under fairly constant external conditions, with an object like a segment of intestine. A distinct initial inhibition was, therefore, caused by serum III, and this was absent with V or VI. The inhibition was followed by increase of tone much less than that produced by the supposedly indifferent sera V or VI.

A point of considerable interest is that the inhibition caused by serum III was distinctly more marked than when that serum was tested in the same way three hours after the withdrawal of the dog's blood, necessarily, of course, on an intestine preparation of another rabbit. This suggests that the pressor effect of a serum may diminish more rapidly on keeping than the adrenalin effect.

In figures 27 and 28 the effect of serum II on the intestine preparation used for figure 26 is shown. At 10 the Ringer was replaced by serum II, and after washing the preparation, the experiment was repeated exactly at 12. There was again in both observations evidence of inhibition preceding the moderate increase of tone. At 14 serum I replaced Ringer and caused an increase of tone without preliminary inhibition. Although serum I is an indifferent serum, it is perhaps worth while pointing out once more that its power of raising the tone of the intestine preparation is very much less than that of another indifferent serum (serum IV) from the same animal, as is shown very well in figure 28, where at 16 Ringer was replaced by serum IV. The magnification was the same as for figure 27 and the experiment was done in precisely the same way.

In figure 29 is illustrated the influence on the uterus of the sera of January 22, tested four hours after the withdrawal of the blood. The rabbit was fully grown or nearly so, but not pregnant. The uterus preparation weighed 0.33 gm. Ringer was replaced at 34 by serum II, at 37 by serum III, at 39 by serum I, and at 42 by serum IV. At 50 observation 37 was repeated.

The fact that serum III caused a much greater increase of tone of the uterus than the indifferent serum IV, while on the intestine (figures 27 and 28) serum IV had a much greater pressor effect than serum III, strengthens the inference that the smaller effect of III for the intestine was due to partial neutralization by adrenalin.

For serum II the preponderance of effect on the uterus preparation, although evident, was not so striking. It seems probable that serum II also contained some adrenalin. Nevertheless, for reasons alluded to, one would hesitate to use this observation to confirm the view that the adrenal continually gives off adrenalin.

At 44 (figure 29) a solution of adrenalin in Ringer (1 : 50,000,000) replaced the Ringer in which the uterus preparation was beating. There ensued a distinct inhibition, after which the curve rose little, if at all, beyond the initial height. At 55 adrenalin (1 : 500,000) in Ringer replaced the Ringer, and a marked increase of tone occurred, preceded by some inhibition. Yet even with this relatively strong adrenalin the increase of tone was not nearly so great as that caused in observations 37 and 50 by serum III, nor did it last nearly so long.

A purely inhibitory effect is not infrequently produced in the virgin rabbit uterus by dilute adrenalin, while stronger solutions cause the opposite effect. Figure 34 shows a still better exampie.

When the adrenalin solutions are made very dilute, a similar apparent reversal of the adrenalin action is sometimes seen with the rabbit intestine, as has been recently pointed out by Hoskins.[13] An instance is given in figure 29, where at 15 adrenalin 1 : 50,000,000 replaced Ringer. The tone was increased and also the strength of the beats, but they diminished after washing (16). That this effect was not due merely to the replacement of Ringer's solution, in which the preparation had long been beating, by a fresh solution was shown by control experiments. A slight, transient increase of tone and of the beats may be caused by changing the Ringer, but these changes are less decided than with the adrenalin solution.

*Experiment of January 19, 1912.*—At 9:29 A. M. a dog weighing 7.9 kilos was etherized and carotid and tracheal cannulæ were inserted. The left great splanchnic nerve was exposed, and a cannula inserted into the transverse lumbar vein just external to the adrenal gland. A ligature was then applied loosely on this vein between the gland and the vena cava. An attempt was at once made to collect a sample of blood from the vein by connecting a small syringe with the cannula, meanwhile tightening the ligature between the adrenal and the vena cava. However, the blood clotted and the syringe was removed. The blood was then allowed to gather in the pocket of peritoneum and was sucked up, with a pipette, as quickly as it collected (sample A). It was observed particularly that the blood did not come into contact with the adrenal. Then the splanchnic was stimulated while a blood pressure tracing was being taken from the carotid. The stimulation was quite effective with regard to blood pressure. During stimulation, blood was collected partly directly from the vein, partly from the peritoneal pocket (sample B), care being taken to prevent it from coming into contact with the gland. This method of collection was not considered satisfactory, but as it had been used by others, it was thought better to continue the experiment in this way rather than to abandon it. In the other experiments, blood was collected from a cannula. Within two hours intestine and uterus tests were begun. The rabbit was a young adult in early pregnancy. The intestine preparation used for figure 30 weighed 0.42 gm. Two uterus preparations weighing respectively 1.85 gm. and 1.51 gm. were employed.

[13] R. G. Hoskins, *Am. Jour. Physiol.*, 1912, xxix, 363.

In figure 30 at 4 serum B, and at 7 serum A replaced Ringer. In both cases there was some initial inhibition followed by a moderate increase of tone. At 10 adrenalin in Ringer (1 : 50,000,000) replaced Ringer, causing a slight diminution of tone. At 18 adrenalin in Ringer (1 : 500,000) replaced Ringer and produced marked inhibition. On the uterus preparation both sera caused very decided increase of tone. Accordingly, the rather moderate increase produced in the intestine does not indicate any deficiency in the normal pressor power for smooth muscular structures, and this is consistent with the conclusion that in the intestine observations the pressor effect is partly masked by adrenalin. But still less than that of January 22 does this experiment yield decisive evidence of adrenalin blood from the gland in the absence of splanchnic stimulation.

## ADRENALIN IN PATHOLOGICAL SERA.

As regards the clinical cases, no lengthy description is necessary, for in no instance was there any indication of adrenalin. The cases included:

A. A man, aged 21 years, with acute cardiorenal disease. Systolic pressure 174.

B. A man, about 30 years old, with Ménière's disease associated with lues, and with chronic tuberculosis of the lungs.

C. A woman with lues who had a much enlarged liver and marked jaundice. The serum was tinged with bile pigment and gave a Gmelin reaction.

D. A man with aortic insufficiency, greatly thickened brachial, forearm, and femoral arteries, a systolic pressure of 190 in the femoral, and 140 in the brachial, and a diastolic of 90 in brachial and femoral, in the horizontal position.

E. A woman with general paresis associated, at the time the blood was obtained, with acute mental disturbance. The blood was drawn during a veritable emotional storm, a condition supposed to be accompanied by liberation of adrenalin.

F. A patient with luetic periostitis.

G. A mulatto man, aged 42 years, with Graves' disease.[14] The symptoms were typical. The diagnosis of Graves' disease was made independently by Drs. Hoover, Phillips, and Marine. The systolic blood pressure was 170, the diastolic 90. The man complained of nervousness and general muscular weakness. He had been suffering for nine months, and had been getting progressively weaker. On examination there was no emaciation. The eyes were distinctly prominent, the pupils were dilated, and the heart rate averaged 120 over a period of four days. There was slight thyroid enlargement (symmetrical), distinct tremor, marked muscular weakness without corresponding loss of muscle volume, capillary and venous pulse in hand, very dry and brittle hair, and a trace of albumen in the urine. Slight enlargement of tonsils, and distinct substernal dullness occupying region of thymus. Splenic area of dullness increased, but spleen not palpable.

P. An elderly woman in whom a diagnosis of myxedema was made.

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[14] This case is described in some detail, as Graves' disease is the condition *par excellence* in which writers on this subject claim to have found adrenalin in the blood. The patient was observed at Lakeside Hospital, to the staff of which and especially to that of the City Hospital, I am much indebted.

H. A woman, 51 years old, with a systolic blood pressure of 290, aortitis, and enlarged heart,—probably with chronic myocarditis and slight chronic interstitial nephritis. The Wassermann reaction was positive.

I. A patient with cerebral apoplexy and a systolic blood pressure of 190.

P. An elderly woman in whom a provisional diagnosis of myxedema was made.

S. A man suffering from an acute attack of gout.

V. A man, aged 26 years, with Vincent's angina but otherwise normal.

W. A woman with pneumonia. The Wassermann reaction was positive.

At 5, figure 31, serum obtained by venesection from case A, replaced Ringer. What appears to be an inhibitory effect preceded the rise of tone. The curve is reproduced to illustrate the point that mere inspection of a single curve like this is not convincing with regard to the detection of adrenalin in serum. For the serum at the date of this experiment was already five days old and if adrenalin in detectable amounts still persisted, the original adrenalin content must have been very great. Yet, when tested two days before, there had been no trace of adrenalin reaction. It is probable that the dip after 5 is due to some variation in the activity of the preparation unconnected with the addition of the serum as such, possibly to the momentary contact of a liquid as yet relatively poor in oxygen. That the preparation at this point was very sensitive to changes in the oxygen supply was shown by the fact that an increase of oxygen at 6 coincided with a very marked fall in the curve, which might have easily been mistaken for an adrenalin effect if it had occurred when the oxygen supply was not being controlled. Then the tone rapidly increased, to a maximum much above that obtained before 6.

The pressor effect of this serum on the intestine was still conspicuous ten days after the blood was drawn, but then there was no trace of an effect like that of adrenalin. At 27 (figure 31) an adrenalin solution in Ringer (1 : 1,000,000) replaced Ringer and caused pronounced inhibition. At 33 the serum replaced Ringer in contact with the uterus preparation. The increase of tone was at least as great as that caused by adrenalin in Ringer (1 : 500,000) when it replaced Ringer at 38. This, let it be repeated, merely shows that the serum preserved considerable pressor power for smooth muscle.

Figure 32 illustrates the reaction of one and the same intestine preparation to three pathological sera. As usual in each observation the segment was initially beating in Ringer. At 2 this was displaced by serum from C. This serum was especially interesting since it was obviously rich in bile constituents. Yet its action on the intestine preparation differed in no way from that of any other serum from the peripheral circulation. At 9 Ringer was replaced by B's serum, at 11 by P's. There is no suggestion in any of the curves of adrenalin action. The effect of a 1 : 10,000,000 solution of adrenalin in Ringer is shown at 4, and that of a 1 : 15,000,000 solution at 14.

Figure 33 shows uterus reactions with the same three sera. The rabbit was nearly full grown and not pregnant. At 19 C's serum replaced one half of the Ringer. At 21 C's serum replaced all the Ringer. In both observations the serum caused decided inhibition with diminution of tone. The effect was distinctly greater with the larger dose of serum, but the curve returned sooner to its original level or even a little above it. On washing with Ringer, the curve fell somewhat (22). At 23 B's serum, and at 25 P's serum, replaced Ringer completely, and at 26 the preparation was again beating in Ringer after washing. On this uterus preparation, then, all three sera caused distinct inhibition followed by restoration of tone to normal or above and by some quickening of the beats. The effect of adrenalin on the same preparation is shown in figure 34. At 28 Ringer was replaced by 1 : 50,000,000 adrenalin in Ringer, at 29 by 1 : 15,000,000, and at 33 by 1 : 1,000,000.

This is a striking example of the opposite effects caused on the uterus by strong and weak solutions of adrenalin, the weaker solutions producing a certain amount of inhibition, and the strong solution (1 : 1,000,000) a great increase of tone with no preceding inhibition. It is certainly impossible from the reactions illustrated to deduce the presence of adrenalin in any of these sera.

Figure 35 illustrates the intestine reaction with serum from S. At 2 Ringer was replaced by the serum. At 4 after the preparation had been washed, the same amount of serum was again added. The tone increased, but diminished again much sooner than in observation 2. The cause of this was deficient oxygenation, as the cylinder became exhausted. At 5 the oxygen was increased from a fresh cylinder and the curve promptly rose, eventually to the height shown in 6. An interval of three minutes before 6 is omitted.

Had the cause of the small rise and rapid fall in observation 4 not been ascertained, and had this been the only observation with the serum, it might have been put down as a serum rich in adrenalin. When observation 17 was made, the same preparation was contracting with great regularity in Ringer. At 17 the Ringer was replaced by the serum. The beats were strengthened but no change in tone occurred. At this time the tone of the preparation was apparently incapable of being altered by any of the sera added or by adrenalin, while the force of the beats could still be markedly affected, as shown in figure 36. This, a rather unusual condition in our experience, is worthy of note apart from its bearing on adrenalin testing, where, if not taken account of, it might lead to fallacy.

In figure 36, with the preparation of figure 35, Ringer was replaced at 9 by D's serum, at 15 by E's, at 12 by adrenalin in Ringer (1 : 5,000,000), and this at 13 by adrenalin in Ringer (1 : 2,500,000). The beats became smaller for a time under the influence of the adrenalin solutions without change of tone, the exact opposite of the effect produced by the sera. It is interesting to note that at 7, before the preparation had lost the power of altering its tone, the replacement of Ringer by adrenalin in Ringer (1 : 5,000,000) caused a marked diminution of tone. There is no evidence from any of these curves that the sera in question contained adrenalin in sufficient amount to be detected. The uterus tests were also negative.

The last tracings we shall refer to are in figure 37, illustrating intestine tests of serum from G. In addition to testing the effect of the serum when it replaced Ringer directly, it was compared with such other human sera as were available by causing it to replace these. At 2 Ringer was replaced by serum from case V, and this at 3 by serum from G. A further increase of tone occurred, with no preliminary inhibition whatever. Exactly the same result was seen when at 6 the serum of G replaced that of W, which was substituted for the Ringer at 5. At 21 Ringer was replaced directly by the serum of G. The curve rose abruptly without trace of inhibition and then more slowly to the height indicated by 22. Uterus tests were equally negative.

### RESULTS.

1. Tested with segments of rabbit intestine and uterus, none of the pathological sera yielded evidence of the presence of adrenalin.

2. Serum from the adrenal veins of the dog collected during massage of the gland gave a distinctly positive reaction, while serum previously collected with the minimum of mechanical and circulatory disturbance of the gland gave a negative reaction.

3. Serum from the adrenal veins without precautions to avoid disturbing the gland sometimes gave positive, sometimes negative tests.

4. Serum from blood obtained without the least disturbance of the glands, from a pocket of the inferior vena cava which received only blood from the adrenals and from the transverse lumbar veins crossing them, gave no evidence of adrenalin except during stimulation of the splanchnics when the reactions for adrenalin were positive.

5. Serum of blood collected from the vena cava of dogs by a catheter pushed up through one femoral vein until the orifice was just anterior to the openings of the adrenal veins, yielded no evidence of the presence of adrenalin whether the splanchnics (one or both) were stimulated or not, any adrenalin contributed by the glands during stimulation of the splanchnics being too much diluted.

## EXPLANATION OF PLATES.

All the tracings are to be read from left to right. Time is marked in half minutes. At the beginning of each observation, unless otherwise stated, the preparations were always beating in Ringer's solution, serum or adrenalin solution, if previously employed, having been carefully washed away with Ringer. Except when otherwise mentioned, the various sera were tested as soon as they could be separated from the blood, as a rule within three or four hours. The larger apparatus, holding 4.5 c.c., was used for the observations illustrated in figures 3 to 12, and in figure 31; the smaller, holding 2.5 c.c., was used for the rest. Unless a statement is made to the contrary, the liquid used for any particular observation was sufficient to replace completely that in which the preparation was initially beating. The tracings have all been reduced one half.

### Plate 77.

Fig. 1. Intestine segment beating in Ringer. At 6 the oxygen was increased.

Fig. 2. Intestine contracting in Ringer. At 33, 1 c.c. of serum was introduced. At 34 an additional 2 c.c. of the same serum was added.

Fig. 3. Intestine. At 31 adrenalin solution 1 : 10,000,000 in calf serum was added. At 33 adrenalin 1 : 10,000,000 in Ringer replaced Ringer.

Fig. 4. The same preparation that was used for figure 3. At 29 adrenalin 1 : 5,000,000 in the calf serum replaced Ringer.

Fig. 5. The same preparation that was used for figure 4. At 25 calf serum, at 23 and 27 adrenalin 1 : 25,000,000 in the calf serum, and at 20 adrenalin solution 1 : 25,000,000 in Ringer replaced Ringer.

Fig. 6. The same preparation that was used for figures 3 to 5. At 35 adrenalin 1 : 50,000,000 in the calf serum, at 37 the serum itself, and at 39 adrenalin in Ringer 1 : 50,000,000 replaced Ringer.

Fig. 7. Intestine. A fresh preparation from the rabbit used for figures 3 to 6. At 4 calf serum, at 7 adrenalin 1 : 5,000,000 in the serum, at 9 adrenalin 1 : 5,000,000 in Ringer, and at 11 adrenalin 1 : 5,000,000 in the serum replaced Ringer.

Fig. 8. Uterus. At 5 adrenalin 1 : 25,000,000 in calf serum, and at 7 adrenalin 1 : 25,000,000 in Ringer replaced Ringer. At 6 the oxygen was increased.

Fig. 9. The same preparation that was used for figure 8. At 10 adrenalin solution 1 :50,000,000 in calf serum, and at 12 adrenalin solution 1 :50,000,000 in Ringer replaced Ringer.

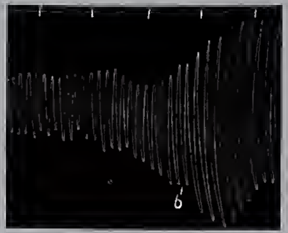


Fig. 1.

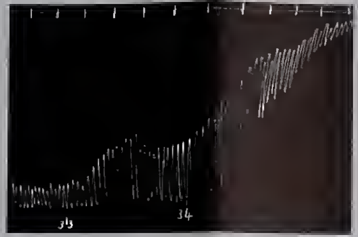


Fig. 2.

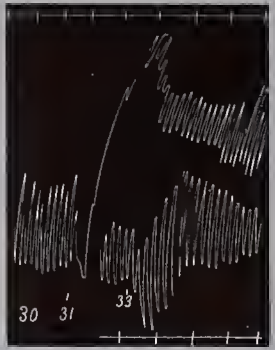


Fig. 3.

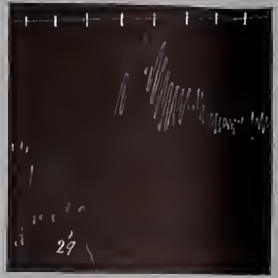


Fig. 4.

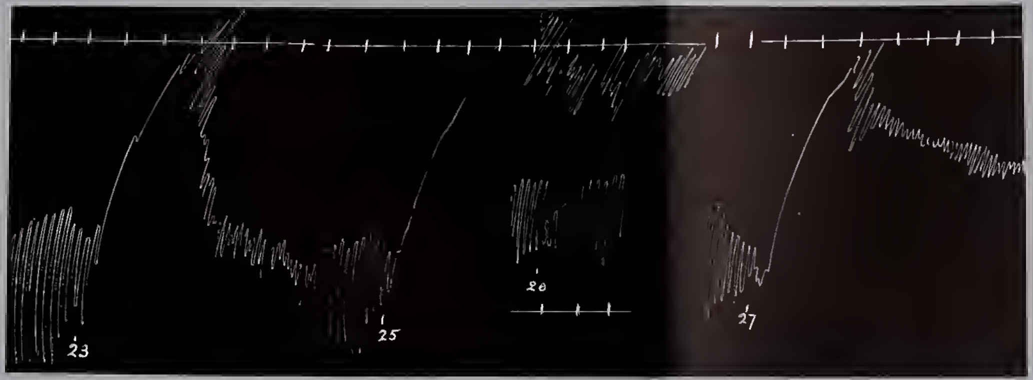


Fig. 5.

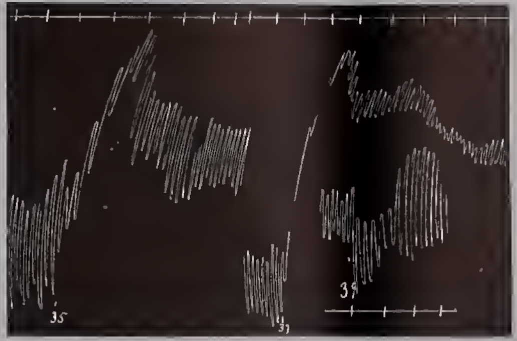


Fig. 6.

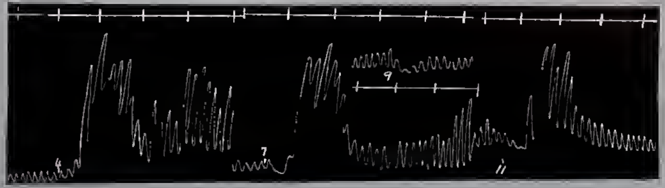


Fig. 7.

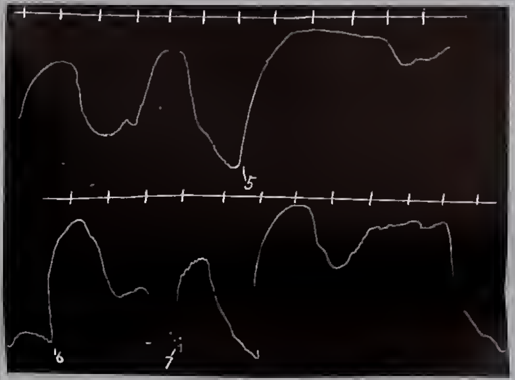


Fig. 8.

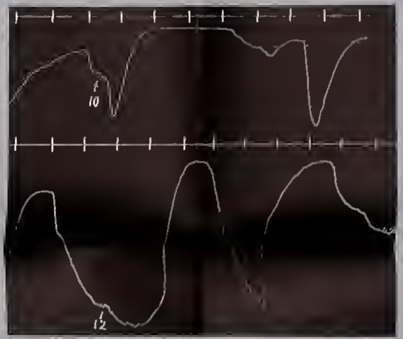


Fig. 9.

## PLATE 78.

FIG. 10. The same preparation as in figure 8. At 14 adrenalin in calf serum 1 : 10,000,000, and at 16 adrenalin in Ringer 1 : 10,000,000 replaced Ringer.

FIG. 11. The same uterus preparation that was used in figures 8 to 10. At 18 adrenalin in calf serum 1 : 5,000,000, and at 21 adrenalin in Ringer 1 : 5,000,000 replaced Ringer.

FIG. 12. The same uterus preparation as in figure 11. At 29 adrenalin 1 : 50,000 in serum, and at 34 adrenalin 1 : 50,000 in Ringer replaced Ringer.

FIG. 13. Intestine. Observations 2, 4, and 9 show the effects produced by the replacement of Ringer by serum I, serum II, and serum IV, respectively, of February 13. At 11 adrenalin 1 : 25,000,000 in serum IV, at 13 adrenalin 1 : 12,500,000 in serum IV, and at 15 adrenalin in Ringer 1 : 12,500,000 replaced Ringer.

FIG. 14. The same intestine preparation as in figure 13. At 6 serum III of February 13 replaced Ringer.

FIG. 15. The same preparation as in figure 14. At 17 adrenalin in serum IV of February 13, 1 : 1,250,000, and at 19 adrenalin in Ringer 1 : 1,250,000 replaced Ringer.

FIG. 16. A fresh intestine preparation. At 28 serum IV of February 13 replaced Ringer. At 29 serum III replaced serum IV. At 36 serum IV replaced Ringer, and adrenalin 1 : 12,500,000 in serum IV replaced serum IV at 37. At 40 Ringer was replaced by serum IV, and this at 41 by adrenalin 1 : 500,000 in serum IV.

FIG. 17. Intestine preparation. At 20 Ringer was replaced by serum IV of February 14, and this at 21 by serum III of February 14. At 22 serum III was displaced by serum IV. At 24 serum IV replaced Ringer. At 25 serum IV was replaced by serum II. At 28 Ringer was replaced by serum IV, and this at 29 by adrenalin 1 : 1,000,000 in serum IV. After washing, adrenalin in Ringer 1 : 1,000,000 replaced Ringer at 40.

FIG. 18. Uterus preparation. At 42 Ringer was replaced by serum IV of February 14, and this at 43 by serum III. At 46 serum IV was added to the preparation after washing. At 47 serum IV was replaced by serum II. After washing, serum IV was again introduced at 50, and was replaced at 51 by adrenalin 1 : 1,000,000 in serum IV.

FIG. 19. Intestine. Serum III and serum IV of February 14, after being kept for forty-eight hours in the ice chest. At 52 Ringer was replaced by serum IV, and this at 53 by serum III. At 55 Ringer was replaced by serum IV, and this at 56 by adrenalin 1 : 5,000,000 in serum IV. At 57 adrenalin 1 : 500,000 in serum IV replaced the 1 : 5,000,000 solution. At 59 Ringer was replaced by serum IV, and this at 60 by adrenalin 1 : 1,000,000 in serum IV.

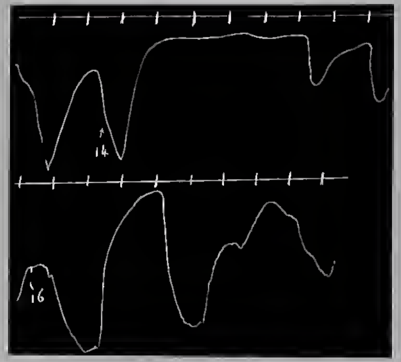


FIG. 10.

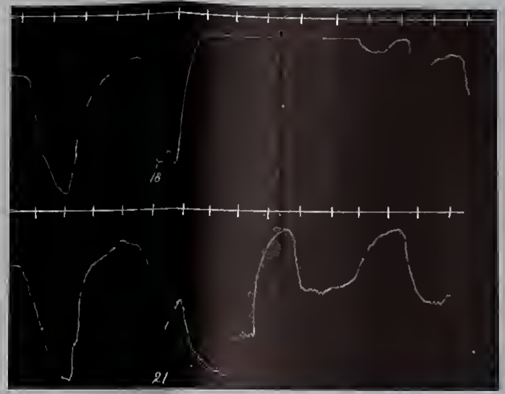
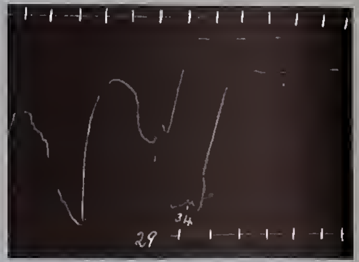


FIG. 11.



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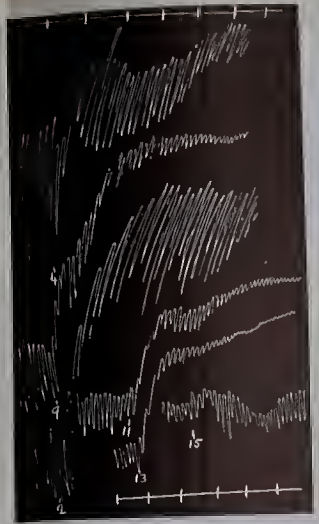


FIG. 13

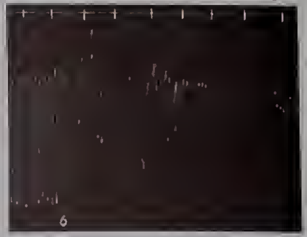


FIG. 14.

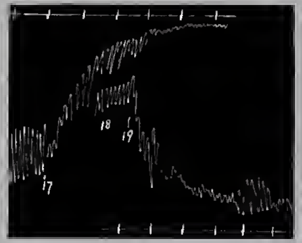


FIG. 15.

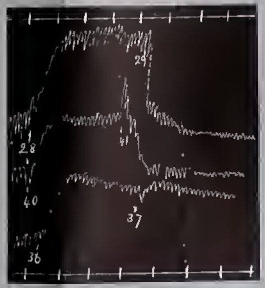


FIG. 16.

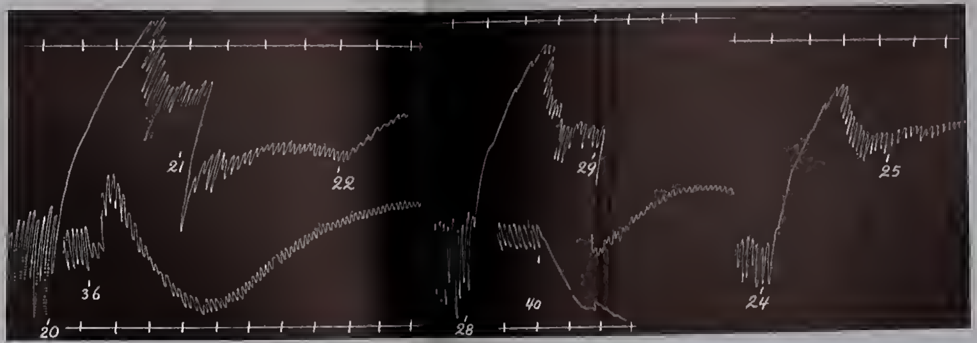


FIG. 17.

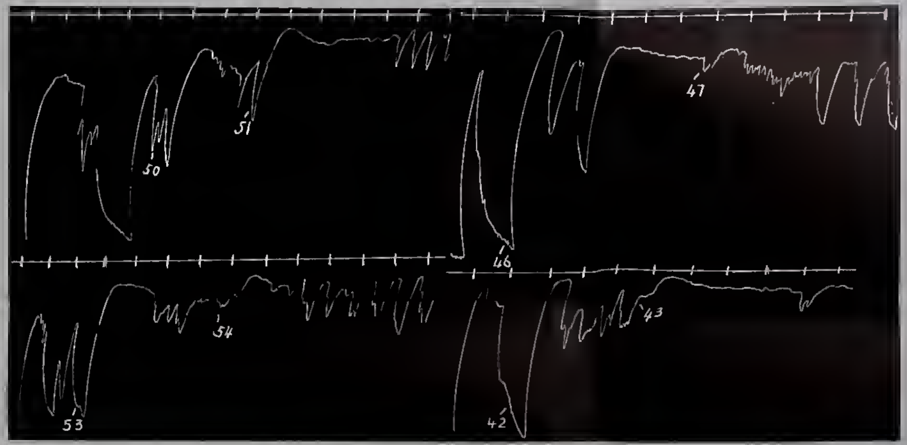


FIG. 18

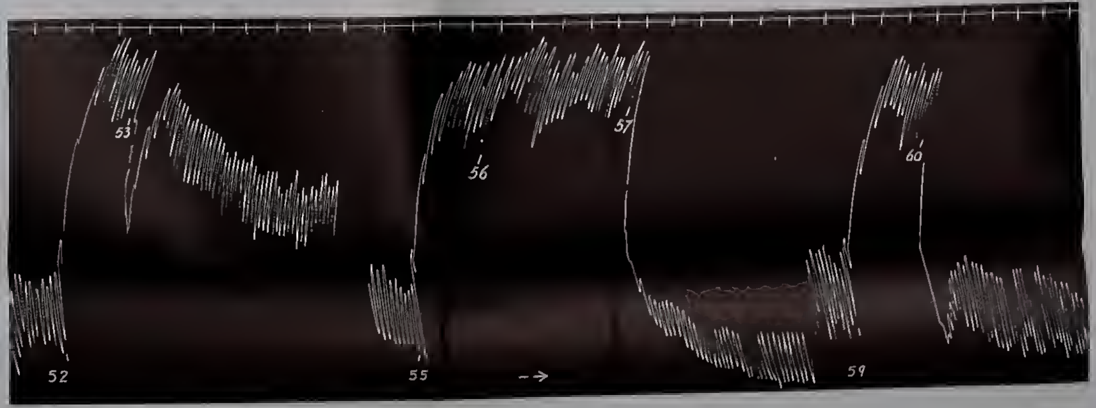


FIG. 19.

## Plate 79.

Fig. 20. Uterus preparation. At 25 Ringer was replaced by serum V of February 16 (drawn twenty-four hours before), and this at 26 by adrenalin 1 : 5,000,000 in serum V. At 28 Ringer was replaced by serum I of February 14 (obtained three days before), and this at 29 by serum III of February 14.

Fig. 21. The same intestine preparation as in figure 19. At 65 Ringer was replaced by serum I of February 16, a few hours after being drawn, and this at 66 by serum III of February 16. At 68 Ringer was replaced by serum I of February 16, and at 69 additional serum I was run into the cylinder. At 70 serum I was replaced by serum III. At 75 Ringer was replaced by serum II, and this at 76 by serum III.

Fig. 22. The same intestine preparation as used in figure 21. At 78 Ringer was replaced by serum VI of February 16 (a few hours after the blood was withdrawn), and this at 79 by serum V. At 49 Ringer was replaced by serum VI, and this at 50 by serum IV. At 81 Ringer was replaced by serum VI, and this at 82 by serum III of February 16.

Fig. 23. Uterus preparation. At 84 Ringer was replaced by serum II of February 16 (a few hours after withdrawal), and this at 85 by serum III. At 92 Ringer was replaced by serum VI, and this at 93 by adrenalin 1 : 1,000,000 in serum VI.

Fig. 24. Uterus. At 9 serum II of February 13 (obtained four days previously), and at 10 serum III of February 13 replaced Ringer. At 2 Ringer was replaced by serum VI of February 16, and this at 3 by serum III of February 16. At 15 Ringer was replaced by serum VI, and this at 16 by adrenalin 1 : 1,000,000 in serum VI. At 22 Ringer was replaced by serum V, and this at 23 by serum III of February 16.

Fig. 25. The same uterus preparation that was used for figure 24. At 5 Ringer was replaced by diluted serum VI of February 16, and this at 6 by diluted serum III of February 16, which at 7 was replaced by diluted serum VI.

Fig. 26. Intestine. At 2 Ringer was replaced by serum VI of January 22, at 4 by serum V, at 6 by serum III of January 22, and at 8 by serum III.

Fig. 27. The same intestine preparation that was used for figure 26. At 10 Ringer was replaced by serum II of January 22. After washing, the experiment was exactly repeated at 12. At 14 Ringer was replaced by serum I of January 22.

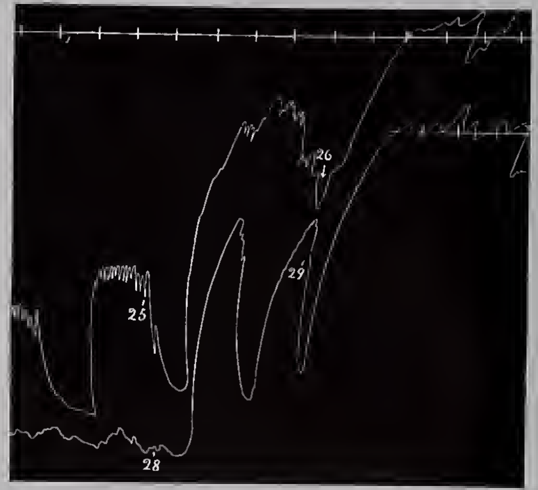


Fig. 20.

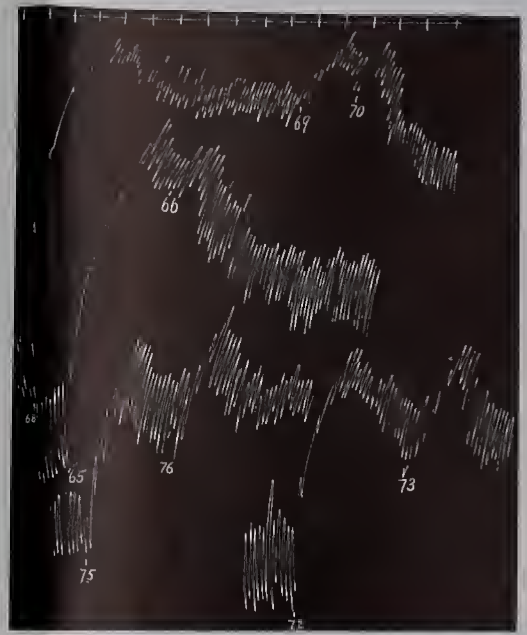
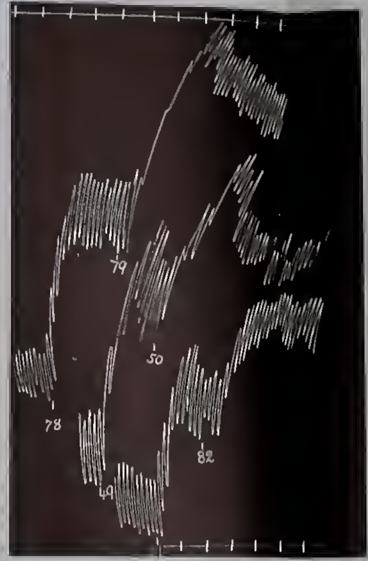


Fig. 21.



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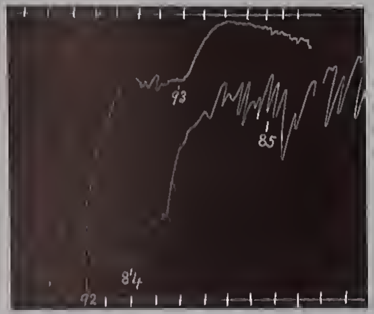
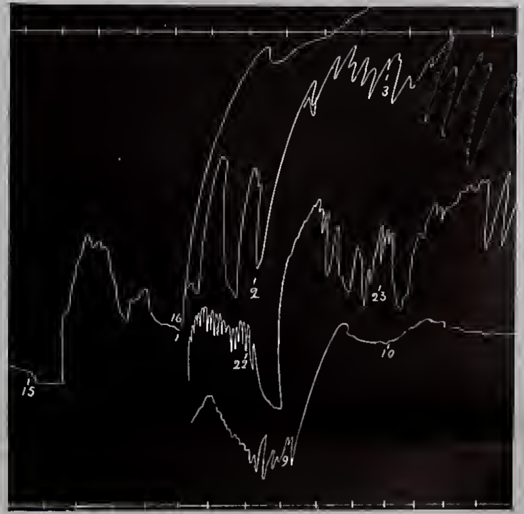


Fig. 23.



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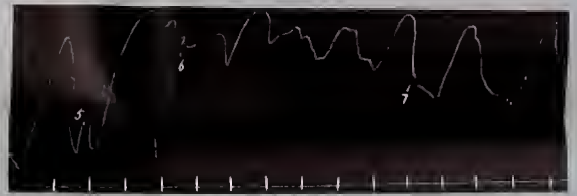

Fig. 25.

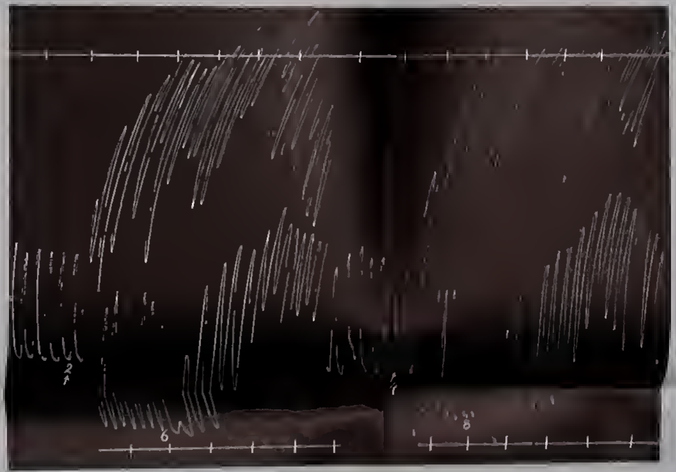

Fig. 26.

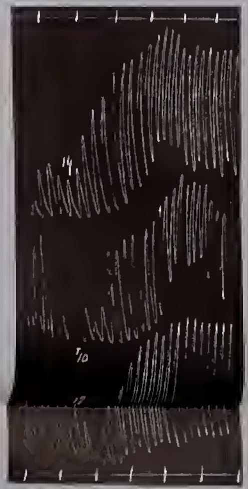

Fig. 27.

## PLATE 80.

FIG. 28. The same intestine preparation as that used for figure 27. At 16 Ringer was replaced by serum IV of January 22.

FIG. 29. Intestine. At 15 Ringer was replaced by adrenalin 1 : 50,000,000 in Ringer, and at 16 the preparation was again beating in Ringer.

Uterus preparation. Sera of January 22 were tested four hours after withdrawal of blood. At 34 Ringer was replaced by serum II, at 37 by serum III, at 39 by serum I, and at 42 by serum IV (the very small elevations on the curve are due to oxygen bubbles). At 50 Ringer was replaced by serum III, at 44 by adrenalin 1 : 50,000,000 in Ringer, and at 55 by adrenalin 1 : 500,000 in Ringer.

FIG. 30. Intestine. At 4 Ringer was replaced by serum B of January 19, at 7 by serum A of January 19, at 10 by adrenalin 1 : 50,000,000 in Ringer, and at 18 by adrenalin 1 : 500,000 in Ringer.

FIG. 31. Intestine. At 5 Ringer was replaced by serum from case A, and at 6 the oxygen supply was increased. At 27 Ringer was replaced by adrenalin 1 : 1,000,000 in Ringer.

Uterus preparation. At 33 Ringer was replaced by serum from case A, at 38 Ringer was replaced by adrenalin 1 : 500,000 in Ringer.

FIG. 32. Intestine. At 2 Ringer was replaced by serum from case C, at 9 by serum from case B, at 11 by serum from case P, at 4 by adrenalin 1 : 10,000,000 in Ringer, and at 14 by adrenalin 1 : 15,000,000 in Ringer.

FIG. 33. Uterus. At 19 half of the Ringer was replaced by the serum of case C. At 21 Ringer was completely replaced by the serum of C, at 23 by serum from case B, and at 25 by serum from case P. At 26 the preparation was beating in Ringer after washing.

FIG. 34. The same uterus preparation as that used for figure 33. At 28 Ringer was replaced by adrenalin 1 : 50,000,000 in Ringer, at 29 by adrenalin 1 : 15,000,000, and at 33 by adrenalin 1 : 1,000,000 in Ringer.

FIG. 35. Intestine. At 2 Ringer was replaced by serum from case S. At 4 after washing, Ringer was again replaced by the serum from S. At 17 Ringer was replaced by serum from S. Note that the tone is now unaltered. The lower time-trace belongs to observation 17.

FIG. 36. At 9 Ringer was replaced by serum from case D, at 15 by serum from case E, and at 12 by adrenalin 1 : 5,000,000 in Ringer, which at 13 was replaced by adrenalin 1 : 2,500,000 in Ringer. At 7, before the preparation had lost the power of altering its tone, Ringer was replaced by adrenalin in Ringer 1 : 5,000,000.

FIG. 37. Intestine. At 2 Ringer was replaced by serum from case V, and this at 3 by serum from case G. At 5 Ringer was replaced by serum from case W, and this at 6 by serum from case G. At 21 Ringer was replaced by serum from case G; 22 in the continuation of the curve. The lower time-trace belongs to observations 21 and 22.

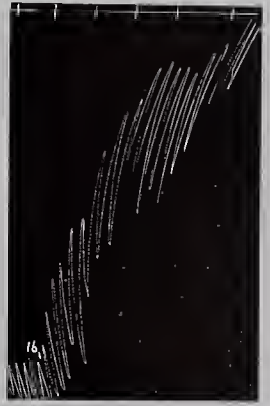

FIG. 28.

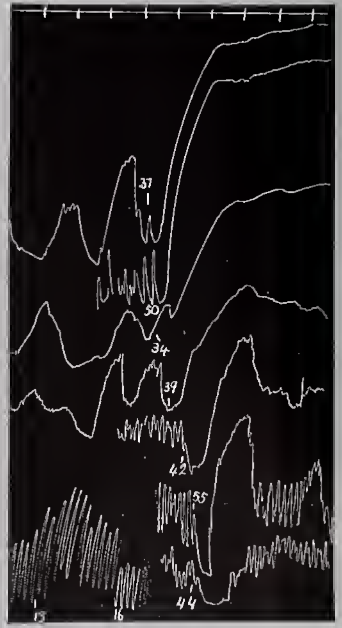

FIG. 29.

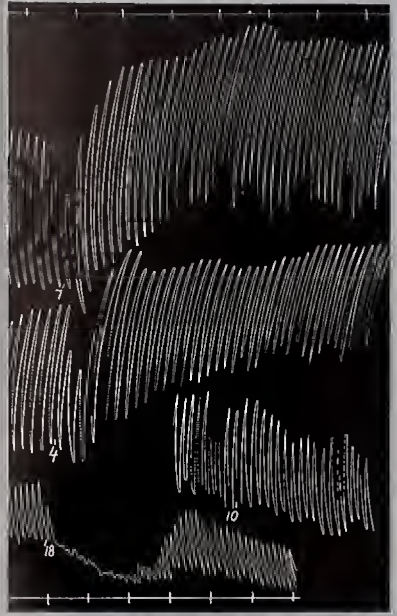

FIG. 30.

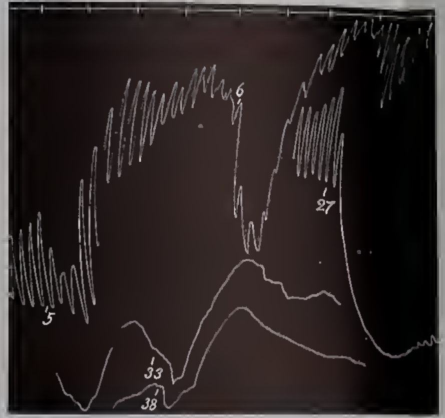

FIG. 31.

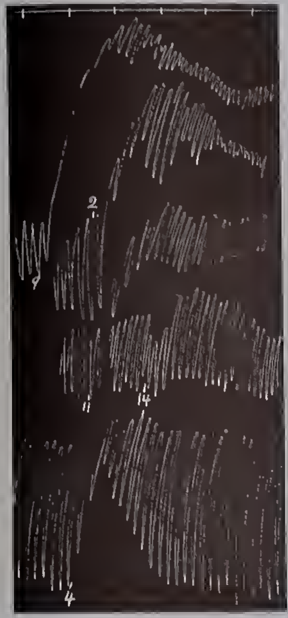

FIG. 32.

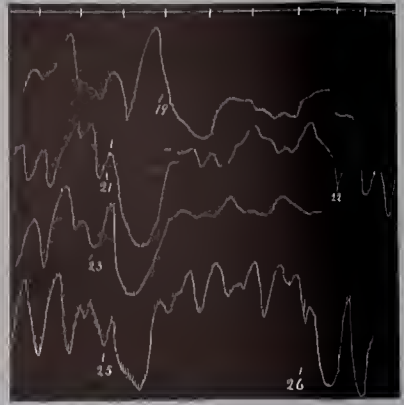

FIG. 33.

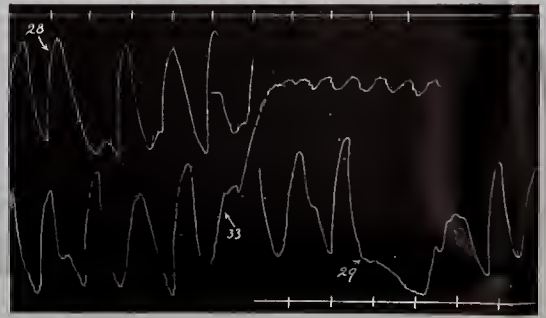

FIG. 34.

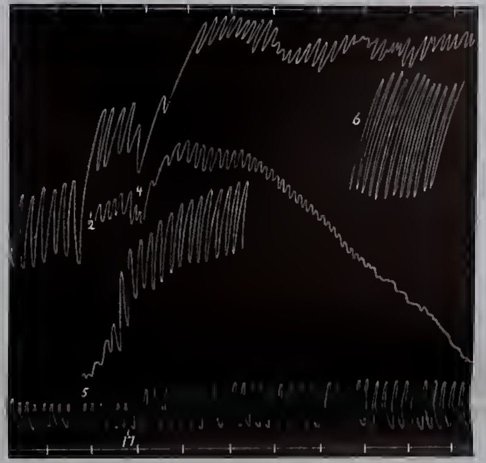

FIG. 35.

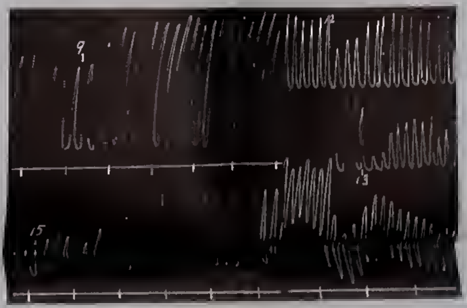

FIG. 36.

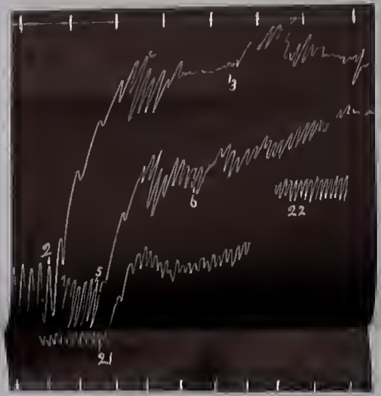

FIG. 37.

# TESTING FOR EPINEPHRIN (ADRENALIN) IN BLOOD. COMPARISON OF PLASMA AND SERUM.

BY G. N. STEWART, M.D.

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# TESTING FOR EPINEPHRIN (ADRENALIN) IN BLOOD. COMPARISON OF PLASMA AND SERUM.*

BY G. N. STEWART, M.D.

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Certain observers have stated that the pressor effect of defibrinated blood or serum is due to substances produced in clotting. If this be so, it is obvious that the complication introduced by this property in testing for epinephrin in blood, which was discussed in a previous paper,[1] can be avoided by preventing the blood from coagulating by the addition of some substance which does not itself interfere with the epinephrin reactions. I have put the question to the test as regards the two objects, intestine and uterus, employed in the previous work, by comparing the action of blood or plasma prevented from clotting by hirudin with the action of the same blood after defibrination or of the serum separated from it. Both dog blood and human blood were used and the result was the same in both cases; that is, that the tone-increasing power of the unclotted blood or plasma for segments of rabbit intestine and uterus was practically identical with that of the defibrinated blood or serum. Possibly in some instances the increase of tone in the intestine produced by the plasma or by the uncoagulated blood was somewhat more transient than that produced by the serum or defibrinated blood. But this is by no means constant and in any case the difference was quite inconspicuous. Serum separated spontaneously from the clot had, as was to be expected, a precisely similar action to that of serum separated by the centrifuge from the defibrinated blood.

It follows from these results that, at least so far as heterologous blood is concerned, no advantage is to be gained by using hirudin blood or plasma instead of defibrinated blood or serum in testing

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* Received for publication, July 1, 1912.

[1] Stewart, G. N., *Jour. Exper. Med.*, 1912, xv, 547.

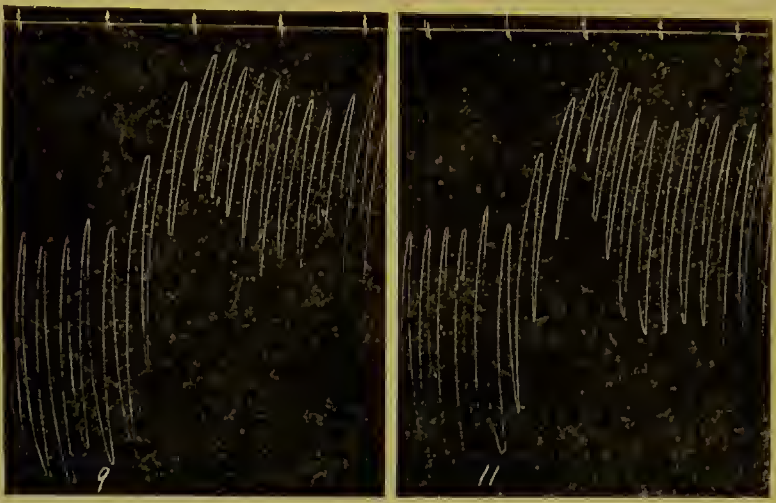
for epinephrin by the rabbit intestine and uterus reactions. A further consequence of these observations is that the substance or substances responsible for the tone-increasing power of serum on intestine and uterus segments initially beating in Ringer's solution are not produced in the process of coagulation but preëxist in the unclotted blood. In proof of these statements the protocols of two experiments, one with dog and the other with human blood, are cited, and specimens of the tracings reproduced.

*Experiment I.*—A solution of hirudin in Ringer's solution containing 0.01 gm. in 1 c.c. was made. The hirudin, just obtained from the importer, was sealed in a glass tube, which was broken to make the solution.

At 11 A. M. 25 c.c. of blood from the carotid artery of a small bitch (under ether anesthesia) was run into 5 c.c. of the hirudin solution. The animal had a large goiter and the flow from the enlarged carotid was very rapid. Some blood was first allowed to escape from the cannula before collection was begun, in order to avoid all risk of the presence of clot. The blood was mixed well with the hirudin solution. No clotting occurred, even in several days. Immediately afterwards, another specimen of blood was collected and defibrinated. Ringer's solution to the amount of one fifth of the volume of the defibrinated blood was added to it so that it should be diluted to the same extent as the hirudin blood. Then the rest of the blood was at once drawn from the animal and allowed to clot after the addition of one fifth of its volume of Ringer's solution. Serum was separated immediately by the centrifuge from the defibrinated blood, and plasma from the hirudin blood. The yield was large and in both cases the liquid was free from blood pigment. The same was true of the serum from the clot, which separated very quickly.

At 2.25 P. M. tests were begun with an intestinal segment of a rabbit. Specimens of the curves are given in text-figures 1 to 3. The smaller apparatus was used, 2 c.c. of liquid being always run into it. The weight of the intestine preparation was 0.46 gm.

At observation 9 (text-figure 1) Ringer's solution in which the preparation was beating was replaced by serum from the defibrinated blood. After washing, the preparation was again beating in Ringer's solution when, at 11, this was replaced by plasma from the hirudin blood. It will be seen that the maximum increase of tone produced by the plasma was almost the same as that produced by the serum. At 13 (text-figure 2) hirudin blood replaced the Ringer and caused a considerable increase of tone. After washing, the preparation was again beating in Ringer's solution when this was replaced, at 15, by the defibrinated blood. The initial tone of the preparation before the addition of the defibrinated blood was less than its initial tone before the addition of the hirudin blood. (The exact relations of the two curves to the base line are preserved in the figure and no change in position of the lever was made between the two observations.) It might, therefore, be expected that if the tone-increasing power of the two specimens was really equal, the absolute increase would be greater with the defibrinated blood, as is seen to be the case. On the other hand, the maximum height is greater for the hirudin blood. In other observations it was shown that where the initial tone of the preparation is the same, the absolute increase of tone produced by hirudin plasma is practically the same as that produced by serum from defibrinated blood.

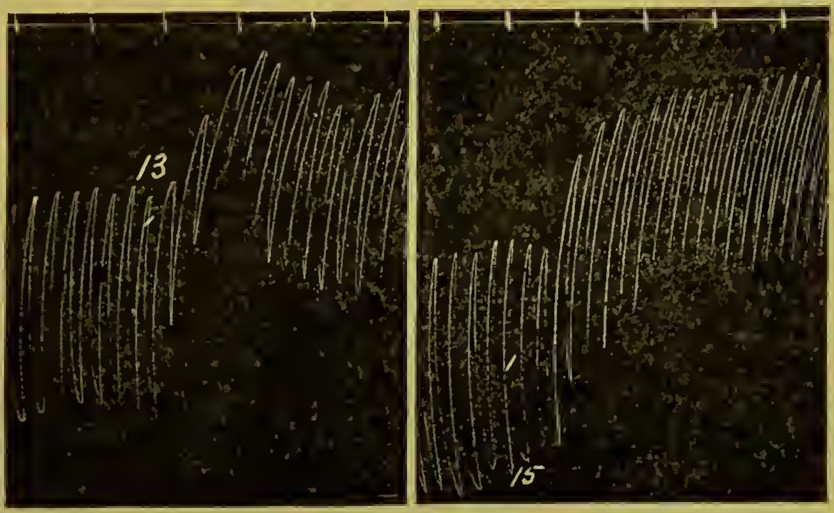


All the tracings are to be read from left to right. Time is marked in half minutes.

Text-figures 1 to 3 were obtained from the same rabbit intestine preparation; text-figures 4 to 6 from an intestine preparation from another rabbit. The magnification of the contractions by the lever was somewhat more than twice as great in text-figures 4 to 6 as in 1 to 3. The smaller apparatus holding 2.5 c.c. without the preparation was used throughout. 2 c.c. of the liquid to be tested, *i. e.*, enough to replace all of the liquid initially present, were always added. At the beginning of each observation the preparations were beating in Ringer's solution, the serum, etc., if previously employed, having been thoroughly washed away with Ringer.

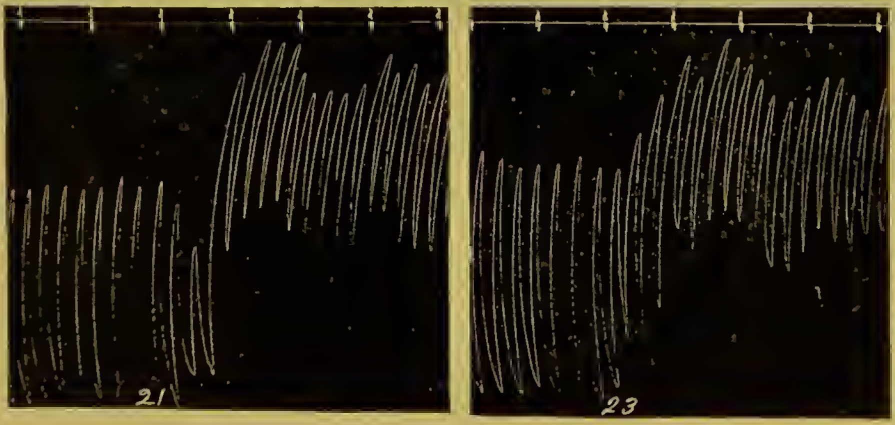
TEXT-FIG. 1. At 9 serum from defibrinated dog blood replaced Ringer. At 11 plasma from hirudin dog blood replaced Ringer.

In text-figure 3 at 21 serum from the clot replaced Ringer's solution. After washing the preparation the Ringer's solution was replaced, at 23, by hirudin plasma. The initial tone of the preparation before 23 was somewhat greater than before 21, and the same remark applies to the comparison that has been made in comparing



Text-Fig. 2. At 13 hirudin dog blood replaced Ringer; at 15 defibrinated dog blood replaced Ringer.

observations 13 and 15. The magnification of the contractions in figures 1 to 3 is somewhat less than half the magnification in text-



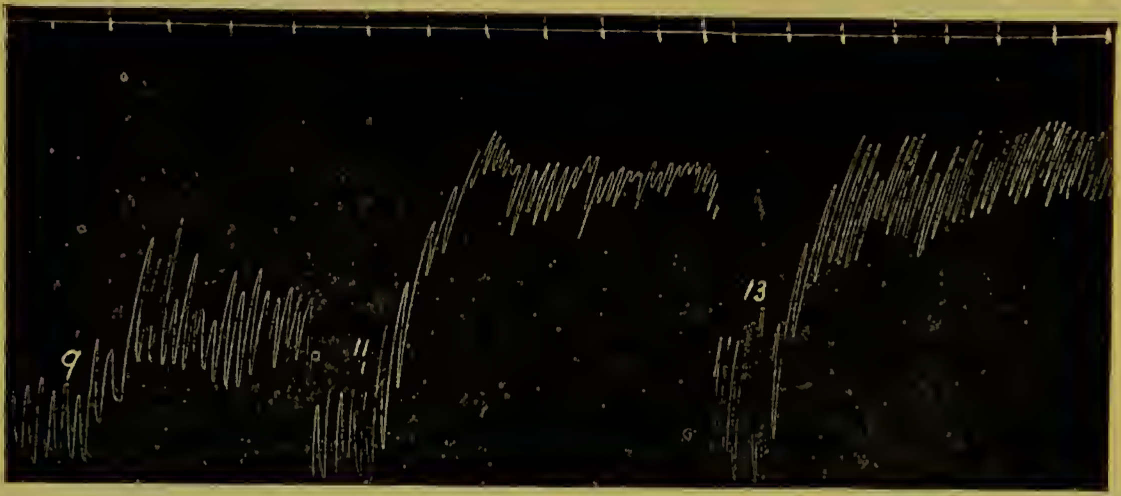
Text-Fig. 3. At 21 serum from clotted dog blood, at 23 plasma from hirudin dog blood replaced Ringer.

figures 4 to 6. The higher magnification was used in most of the experiments in the previous paper. This is pointed out lest the effects in text-figures 1 to 3 might be underestimated by a reader who looks casually at the tracings.

The uterus tests showed no constant difference between the various specimens.

*Experiment II.*—Blood was obtained by puncture of an arm vein from F. W., a man aged 44 years, with mitral insufficiency and a history of lues several years ago.[2]

Just before the vein was tapped, 0.04 gm. of hirudin was dissolved in 4 c.c. of Ringer's solution, and 22 c.c. of blood were then run into the solution. The test-tube was agitated constantly as the blood ran in so as to insure thorough mixture of the blood and hirudin solution. The flow of blood was free. The first portion coming through the cannula was rejected. No clotting whatever occurred, even in several days. As soon as the hirudin specimen had been obtained, 70 c.c. of blood were at once collected from the same man in another vessel, and allowed to clot. 13 c.c. of Ringer's solution were added to it. Both specimens were immediately conveyed to the laboratory and put in the ice chest over night. Abundance of hemoglobin-free serum and plasma separated.



TEXT-FIG. 4. At 9 serum from F. W.'s clotted blood, at 11 his hirudin plasma, at 13 serum from his clot, replaced Ringer.

At 11 A. M. the following morning tests were begun with an intestine preparation from a rabbit. The preparation weighed 0.44 gm. The smaller apparatus was used.

Observation 9 (text-figure 4) shows the effect of replacing Ringer's solution by serum from the clot, and 11 the effect of re-

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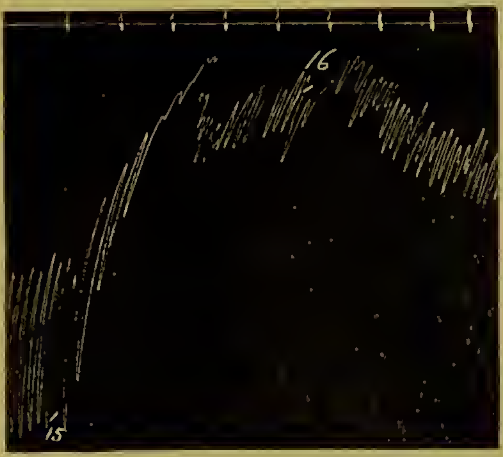
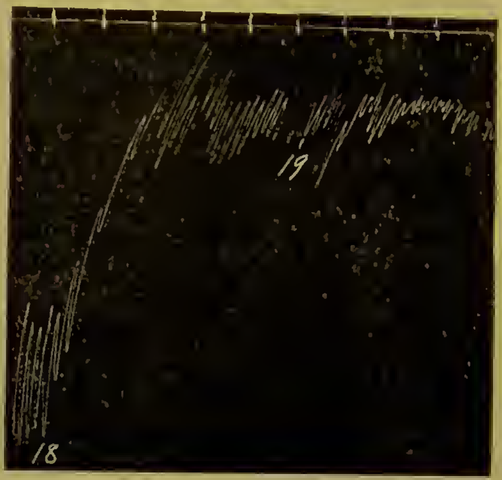
[2] I am much indebted to Dr. Bucher, of the City Hospital, for aiding me in collecting this material. While Dr. Bucher attended to the tapping of the vein. I gave undivided attention to the proper collection of the hirudin blood.

placing Ringer's solution by hirudin plasma. On the face of these two observations alone, the hirudin plasma is so far from being inferior in tone-increasing power that the increase is quite twice as great as that caused by the serum. This, however, is not a fair comparison. For 9 was the first observation in which this intestinal segment had come into contact with serum, and it is sometimes seen that the effect of the first serum bath, particularly in the case of a segment which is not beating very strongly, is to increase the power of response of the preparation to subsequent immersions in serum. This part of the tracing is reproduced here merely to illustrate the point that repeated observations are essential if errors of interpretation are to be avoided in this kind of work. At 13, after washing the preparation, the Ringer's solution was replaced by serum from the clot. The increase of tone is perhaps slightly greater than in observation 11, but the difference is no more than may be found in successive observations with the same serum. At 15 (text-figure 5) Ringer's solution was replaced by hirudin plasma, which at 16 was replaced by serum from the clot. In text-figure 6 this experiment was performed in the reverse order, serum from the clot replacing Ringer's solution at 18 and being itself replaced by hirudin plasma at 19. If the tone-increasing power of the serum had been conspicuously greater than that of the plasma, the rise at 16 ought to



Text-Fig. 5. At 15 Ringer was replaced by F. W.'s hirudin plasma, and this at 16 by serum from his clotted blood.

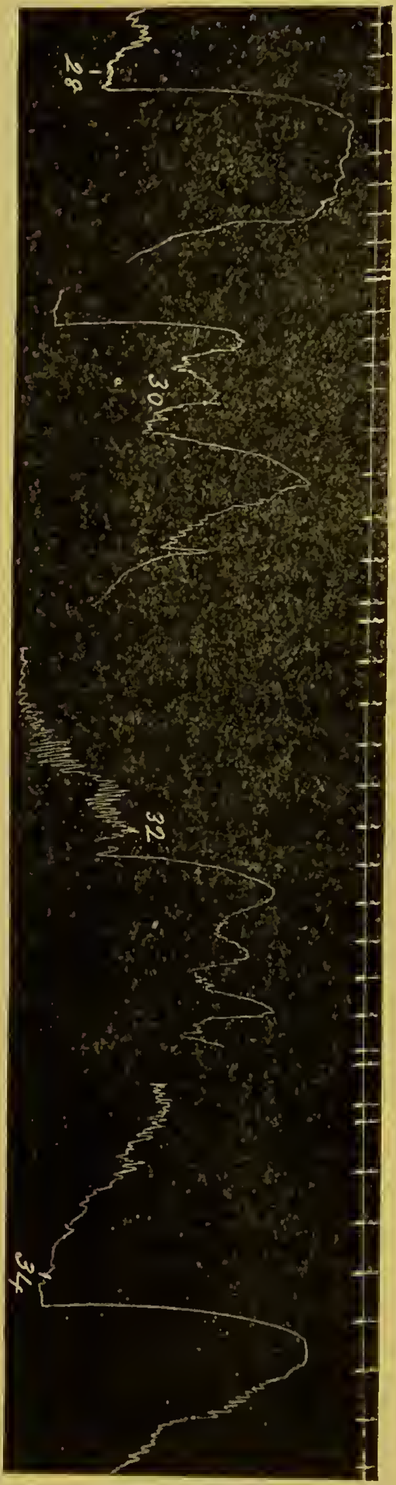
have been decided and sustained, whereas at 19 there should have been a marked fall. On the contrary, there is a slight rise at 19 just as at 16, a rise of the same order of magnitude as is caused by simple renewal of one and the same medium, and the increase of tone after 19 is actually better sustained than after 16.



Text-Fig. 6. At 18 Ringer was replaced by serum from F. W.'s clotted blood, and this at 19 by his hirudin plasma.

Text-figure 7 illustrates the uterus tests with the same specimens of hirudin plasma and serum from F. W. The uterus preparation (a segment of a horn) weighed 0.58 of a gram. The rabbit was a young adult, not pregnant. The smaller apparatus was used.

At 28 hirudin plasma replaced Ringer's solution. A great increase of tone ensued. After washing, the preparation was again beating in Ringer's solution when, at 30, serum from the clot was run in. Starting from a considerably higher level, the absolute increase of tone is not so great as that produced by the plasma at 28, but is nevertheless quite distinct. At 32 hirudin plasma replaced Ringer's solution at a point corresponding to a somewhat greater tonic contraction of the segment than before observation 28. The rise, while considerable, is less than in 28. At 34, after washing, the tone of the segment was less than in any of the previous observations and the addition of serum was followed by a rise of about the same absolute amount as that caused by the plasma in observation 28.



Text-Fig. 7. Uterus preparation. At 28 Ringer was replaced by F. W.'s hirudin plasma, at 30 by serum from his clot, at 32 by his hirudin plasma, at 34 by serum from his clot. After each observation the preparation was thoroughly washed with Ringer.

Certainly in those observations there is nothing to support the idea that substances capable of increasing the tone of the uterus are produced in blood during coagulation. If anything, the plasma caused a greater and more persistent increase of tone than the serum. The difference, however, lay within the limits of variation in such observations even with one and the same liquid.

I have no experience of the perfusion method introduced by Läwen and developed by Trendelenburg, and I am, therefore, not in a position to discuss O'Connor's[3] statement that the pressor effect on perfused frog blood-vessels is almost entirely absent if the blood has been prevented from clotting. He says that the same is true of the tone-increasing power on the rabbit intestine and uterus. He worked, however, in the case of the intestine and uterus with homologous blood. From the point of view of this paper, only the action of heterologous serum and plasma is of interest, since in clinical testing the blood must always be heterologous to the test object. My experiments, therefore, do not strictly compare with O'Connor's. It is, nevertheless, noteworthy that he makes the following statement:

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[3] O'Connor, J. M., *Arch. f. exper. Path. u. Pharmakol.*, 1912, lxvii, 195.

> "Es ist aber keineswegs leicht, in allen Fällen sicher reines Plasma zu gewinnen und die Resultate fallen nicht eindeutig aus, wenn bei der Gewinnung des Plasmas nicht jeder Zellzerfall vermieden wird; aus diesen Grunden ist es auch schwierig, die Unwirksamkeit des Hirudinplasmas auf Darm und Uterus zu erweisen; bei dem Zusammenbringen des Plasmas mit den in Ringerlösung suspendierten Organen entsteht durch die Anwesenheit der Thrombokinasen in den Geweben leicht Gerinnung. Dennoch ist es mir gelungen, sowohl am Darm als am Uterus einwandfreie Versuche zu erhalten."

I can only say that in my experiments every care was taken to collect the hirudin blood under the best conditions. The large dose of hirudin was purposely chosen to insure the absence of the slightest trace of clotting; also, as already mentioned, the flow of blood was very free. Not the slightest indication of clotting was seen at any stage of the experiments. Plasma which had once come into contact with the intestine or uterus preparation was never used a second time. The almost exact quantitative agreement in the action of the plasma and serum appears to me to be a decisive argument against the possibility that the effect of the plasma was due to a certain amount of "cell destruction" which had not been avoided. Had the plasma exerted an effect always conspicuously smaller than that of the serum such a possibility might have been entertained, but it is difficult to see how the effect could be the same unless it is independent of clotting. That occasional instances may occur in which hirudin blood or plasma apparently causes a relatively slight increase of tone while defibrinated blood or serum causes a large increase may be granted. But the converse observation may also sometimes be made, and in interpreting such results the fact that the intestine or uterus preparation varies from time to time in its power of response to one and the same liquid must not be forgotten. For instance, as already pointed out, in text-figure 4 the first two observations (9 with serum, and 11 with plasma), if standing by themselves, would indicate that the tone-increasing power of the serum was much less than that of the plasma, a conclusion which certainly would be erroneous. Suppose that one had happened at 9 to add plasma instead of serum, there is scarcely any doubt that practically the same curves as were actually written would have been obtained. Had the experiment stopped here the interpretation might have been put upon it that hirudin plasma has a far smaller tone-increasing power than serum, and this conclusion would also have been unjustified. Observation 9, as was shown by subsequent tracings, is an anomaly in the series and must not be used in the comparison. If it were used, it would permit precisely the opposite conclusion to that which O'Connor deduced from his observations. These remarks are far from being intended as a general criticism of O'Connor's interesting paper. His conclusion that epinephrin cannot be demonstrated with certainty in the peripheral venous and arterial blood agrees entirely with that drawn in my previous papers.[4] Even if it be admitted, as O'Connor says it must be, that it is difficult to avoid errors of technique in obtaining plasma suitable for use in the intestine and uterus reactions, this alone is sufficient to show that in epinephrin-testing for clinical purposes by means of these reactions no advantage can be gained by endeavoring to substitute plasma for serum.

[4] Stewart, G N., *Jour. Exper. Med.*, 1911, xiv, 377; 1912, xv, 547.

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# A COMPARISON OF THE ACTION OF PLASMA AND SERUM ON CERTAIN OBJECTS USED IN BIOLOGICAL TESTS FOR EPINEPHRIN.*

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PLATES 11 TO 21.

O'Connor,[1] working with citrate plasma on the frog perfusion preparation of Laewen, came to the conclusion that this plasma exerts no constrictor effect on the blood vessels of the frog, while serum to which the same amount of citrate has been added possesses the constrictor action demonstrated by Battelli and others for various kinds of serum.[2] With hirudin plasma and serum applied to segments of intestine and uterus, O'Connor states that the same difference can be demonstrated as regards the tone-increasing effect, plasma being without or almost without action, while the defibrinated blood plus hirudin shows the stimulating action. Yet he admits that it is difficult to obtain faultless experiments on these two objects.

Trendelenburg,[3] working with hirudin material on the frog perfusion preparation, was convinced that the plasma has a constrictor effect which stands to that of the corresponding serum in a definite ratio (1:2½ to 3).

Kahn[4] states that he has confirmed on the frog perfusion preparation the result that hirudin plasma in most cases has a smaller constrictor effect than the corresponding serum. The effect of hirudin plasma, according to Kahn, is much less constant than that of the serum. He does not agree with Trendelenburg that any definite ratio exists between the amount of the effect produced by the plasma and by the serum.

There seems to be a tendency to generalize such results into the conclusion that serum exerts on smooth muscle in general a stimulating or tone-increasing action which is absent from, or feebly developed in plasma obtained in any way whatever. It is clear, however, that a general statement contrasting the action of plasma and serum on smooth muscle organs can be drawn only after a comparison (1) of sera with plasmas obtained in as many different ways

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[1] O'Connor, J. M., *Arch. f. exper. Path. u. Pharmakol.*, 1912, lxvii, 195.

[2] Battelli, F., *Jour. de physiol. et de path. gén.*, 1905, vii, 625, 651.

[3] Trendelenburg, P., *München. med. Wchnschr.*, 1911, lviii, 1919.

[4] Kahn, R. H., *Arch. f. d. ges. Physiol.*, 1912, cxliv, 251.

as possible from one and the same blood on one and the same test object, and (2) of each kind of plasma and the corresponding serum on as large a series of different test objects as possible. There is reason to believe that, within a wide range at any rate, the influence of differences in the species of the animal from which blood on the one hand, and the test objects on the other are obtained is not important.

We attempted a partial survey of this kind by investigating the action of three kinds of plasma, (a) citrate, (b) peptone, (c) hirudin, on four test objects, (a) artery rings, according to Meyer's method, (b) the frog perfusion preparation, (c) intestine, (d) uterus segments. In addition, observations were made on the same objects with certain transudates and exudates. The transudates, for example hydrocele fluid, were chiefly interesting from our point of view as affording the means of comparing serous liquids which certainly had not clotted and had no tendency to clot spontaneously, with serum. They are, for convenience, described in a separate paper,[5] but the results are also used in the discussion in this paper. Some results of interest in connection with the general subject were also obtained by comparing the effect on the different test objects of serum treated in various ways. This work, which is still in progress, will be alluded to briefly here and merely in so far as it bears directly upon the question discussed in this paper. The behavior of some of the test objects to certain foreign proteins was also studied from the same standpoint.

## TECHNIQUE OF OBTAINING THE MATERIAL.

*Citrate Material.*—Citrate plasma was always obtained by the following procedure. A cannula and attached rubber tube were boiled, oiled, and filled with a citrate solution made by dissolving sodium citrate in Ringer to the extent of 2 per cent. After the cannula had been inserted into the carotid of a dog, the end of the rubber tube was dipped below the citrate solution and a volume of blood equal to the citrate-Ringer solution was run in. Then the blood and solution were stirred gently but sufficiently to insure proper mixing. In case the plasma and serum were to be used for perfusion experiments the blood was kept in the incubator for one to one and one-half hours.[6] Each fluid used for perfusion was also filtered before injection. The serum for comparison was derived from defibrinated blood of the same animal to which an equal volume of citrate-Ringer solution was added while the mass of fibrin was still floating in the blood. After mixing the fibrin was removed. In a few experiments we used whole blood for the rings, the intestine, and the uterus preparation, but in the majority we used serum and plasma. For the perfusion experiments, it was of course necessary to centrifugalize repeatedly until all the corpuscles were separated. In addition to citrate serum, serum to which no citrate had been added, usually from the clotted blood, was also generally tested for the sake of comparison.

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[5] Stewart, G. N., and Zucker, T. F., *Jour. Exper. Med.*, 1913, xvii, 174.

[6] Trendelenburg, P., *Arch f. exper. Path. u. Pharmakol.*, 1910, lxiii, 161.

*Peptone Plasma.*—The technique for obtaining this is given in the protocols of the experiments quoted.

*Hirudin Material.*—The technique for obtaining hirudin plasma was practically the same as for citrate plasma. The blood was run into a solution containing 1 mg. of hirudin per cubic centimeter of Ringer solution. The proportion of hirudin solution used varied, and is given in the protocols of the separate experiments. The serum for comparison was obtained by diluting the defibrinated blood with the proper amount of hirudin solution before centrifugalization. The blood was kept in the incubator for a time before separating the plasma and serum if they were to be used for perfusion. In all the experiments except those with the material of September 26 the hirudin plasma was obtained from dogs. The material of September 26 was purposely obtained from rabbits in order to determine whether the use of homologous material made any difference as regards the intestine and uterus preparations.

## TECHNIQUE OF MAKING THE TESTS.

*Vessel Rings.*—The test object consisted of a ring about 1.5 mm. wide cut from a sheep's carotid artery. With practice the width of the rings can be made nearly uniform. In a small cylindrical glass vessel with a capacity of about 2 c.c. the ring was held between two hooks, one fastened to the bottom of the vessel, the other connected with the lever by a light silk thread. The hooks were made of aluminium wire. After the ring was suspended in this way the vessel was filled with Ringer solution, lowered into the bath, kept between 39° and 40° C., and oxygen was bubbled through the solution by means of a side tube near the bottom of the vessel. In the present work the weight used in stretching was 10 gm. The time allowed for stretching was five minutes, this being found practically as effective in producing a sufficient permanent relaxation as a period of fifteen to twenty minutes. Stretching with a heavier weight did not, according to our experience, appreciably increase the sensitiveness of the rings to serum. After stretching, the weight was removed and the preparation allowed to come to equilibrium. Then the fluid to be tested was made to replace the Ringer solution by means of a bent pipette drawn out so as to reach to the bottom of the vessel without disturbing the ring. The ring was allowed to contract under the weight of an ordinary muscle lever, the shortening of the ring being magnified twenty-one times. In most cases when plasma was used which gave no effect or only a slight effect, this was replaced by an active serum. In this case the serum always gave a smaller effect than when used to displace the original Ringer solution. Therefore, for comparison only those curves were used which were obtained in the same way.

*Perfusion.*—In the frog perfusion experiments the technique used was essentially that of Trendelenburg.[7] At first considerable difficulty was experienced in inserting the cannulas. It was found later that this was largely due to the fact that the frogs had been freshly caught. Their vascular sensibility was so great that immediately on cutting the vessels contracted sufficiently to make it well nigh impossible to insert even the smallest cannula. When kept in a tank indoors without food they apparently became much less sensitive in this respect, and after a week or two the difficulty disappeared. Pithing the frog twelve to twenty-four hours before the experiment also rendered the insertion of the cannulas easier. Care must of course be exercised in putting on the ligatures so that leakage is avoided as far as possible. Still, with every care, some oozing of fluid is likely to occur and occasional drops may form and run down from the preparation without passing through the outflow cannula. Since, however, when the preparation is properly made the outflow from the cannula proceeds with great regularity and includes by far the greatest part of the perfused liquid, slight leakage does not seriously invalidate the experiment.

In the experiments in which citrate plasma was used the perfusion fluid contained 0.5 per cent. sodium citrate. But in order to allow clotting to occur as far as possible at the cut surfaces to close up the small vessels which could not be ligated, the perfusion was always begun with ordinary Ringer solution, two Mariotte bottles, one containing Ringer and the other citrate-Ringer, being connected by a T-piece with the perfusion system. It was found advantageous to avoid high pressure in perfusing because the higher the pressure the sooner excessive edema sets in, which diminishes considerably the rate of perfusion. The amount injected and the dilutions are given in the curves reproduced in figures 2 and 3 and in the descriptions of figures 4 and 5. We agree with the observation of Broeking and Trendelenburg[8] that "like all forms of biological testing, the perfusion method gives perfectly constant and satisfactory results only after long experience."

*Intestine and Uterus Preparations.*—The technique was the same as that previously employed by one of us.[9] The smaller apparatus, holding 2.5 c.c. without the preparation, was used throughout. Unless otherwise stated, enough liquid (2 c.c.) was always added to displace the whole of the liquid initially present.

## RESULTS.

### CITRATE PLASMA AND SERUM.

Experiments were made with eight samples of material from different animals.

*Artery Rings.*—The results were practically uniform. With the rings the citrate plasma usually gave a small effect. Where no effect was given the serum also had a relatively small action. Accordingly we recognized as suitable for our purpose only arteries which gave a decided effect with serum. The corresponding citrate serum invariably produced a greater effect than the plasma, and in all the experiments but one a much greater effect.

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[7] Trendelenburg, P., *Arch. f. exper. Path. u. Pharmakol., loc. cit.*

[8] Broeking, E., and Trendelenburg, P., *Arch. f. klin. Med.*, 1911, ciii, 168.

[9] Stewart, G. N., *Jour. Exper. Med.*, 1911, xiv, 377; 1912, xv, 547.

In reporting the artery ring experiments it would be without object to express the results in numbers,—for instance, the maximum rise obtained, or the latent period which elapsed before the rise began, or the amount of the rise which took place in a given time following the change of liquid,—since such numbers would give to our results by this method an appearance of quantitative accuracy which would be fallacious. The following designations will therefore be used. Slight rise, as in figure 1, observation 14; moderate rise, as in figure 1, observation 5; good rise, as in figure 13, observation 18; very good rise, as in figure 1, observation 16. In some cases it was doubtful whether there was any rise of the lever due to the substance added, or whether we were dealing simply with the slow, elastic recoil of the ring which might in any case have taken place. Condensed results of the experiments follow.

*Protocols.*—Aug. 19, 1912. Citrate plasma gave practically no rise; citrate serum, a good rise.

Aug. 22. Citrate plasma gave practically no rise; the corresponding citrate serum gave a good rise.

Sept. 13. Citrate plasma gave a slight rise; citrate serum, a very good rise; serum without citrate, a very good rise.

Sept. 14. The same plasma and serum that were compared on Sept. 13. Plasma gave no noticeable rise; citrate serum (replacing plasma), a good rise.

Sept. 16. Citrate plasma, no noticeable rise; citrate serum, a fairly good rise, which would correspond to something intermediate between moderate and good in the above scheme. Citrate plasma from blood whipped for fifteen minutes before separating the plasma, as was the case with the defibrinated blood used for comparison, gave no noticeable rise. The object of whipping the blood was to test the question whether in this way any pressor effect could be developed when the blood was whipped after some time had elapsed since it had been shed.

Sept. 19. Citrate plasma, no noticeable rise; serum plus citrate, moderate rise; serum from clot, very good rise; serum from clot diluted with an equal volume of Ringer, good rise.

Sept. 21. Citrate plasma, very slight rise (less than would correspond with a slight rise in the above scheme); citrate serum replacing plasma, slight rise; citrate serum replacing Ringer, moderate rise; serum from clot, good rise.

Sept. 24. Citrate plasma, slight rise; citrate serum, good rise; serum without citrate, very good rise.

Oct. 16. The details of this experiment are given in the text (p. 162).

*Perfusion Preparation.*—On this preparation, also, the results were more uniform than with the only other plasma used for perfusion,—hirudin plasma. Whenever serum gave a marked slowing in the flow, the slowing produced by the corresponding plasma was decidedly less. Yet the plasma invariably produced some slowing, and where the serum effect was small, the relative difference between the action of the plasma and serum was less than where the serum effect was large. The smaller the effect produced by the serum, the greater the tendency for the difference between it and the plasma effect to disappear. For this reason only experiments in which serum gave a decided effect were looked upon as available for deciding the question at issue.

Figure 2 gives in the form of curves an example of the results for one specimen of the citrate material. Small portions of the actual drop records are reproduced in figure 4.

*Intestine.*—The intestine preparation, on the other hand, showed no constant difference between the citrate plasma and serum. With each, the first effect was a rapid increase of tone, which, however, was much more transient than that given by plasma or serum not containing citrate. The rise quickly gave place to a diminution of tone which in some cases sank far below the initial level, the strength of the beats being at the same time diminished. There is every reason to believe that the initial increase of tone represents the effect of the plasma and serum as such, and the subsequent depression the effect of the citrate, which more or less completely neutralizes the tone-increasing power of the serum and plasma as indicated by control observations with citrate-Ringer solution. Since the citrate action in the concentration used masks to a great extent the tone-increasing power of the serum or plasma, the comparison on the intestine preparation is not quite so satisfactory as with the other plasmas and sera. Yet there is no evidence of any inferiority of the plasma as compared with serum in its power to increase the tone of this smooth muscle preparation in the presence of the opposite effect of the citrate. Indeed some of the tracings might seem to indicate that the plasma may have an even greater effect than the serum in neutralizing the depressant action of the citrate. We should hesitate, however, to conclude that the plasma is ever superior to the serum in this regard, as it is doubtful whether the differences noted in certain observations do not fall within the normal limits of variation.

*Uterus.*—For the uterus preparation also, the plasma and serum show practically the same behavior. This statement is based on an examination of the citrate material of August 19, September 16. and September 24. Specimen tracings are reproduced from the experiment of September 24 for the intestine (figure 6) and for the uterus (figure 7). In the experiment of September 16 the preliminary rise of tone in the intestine was better marked, in that of August 19 less marked, than in the tracings reproduced both with plasma and with serum. But the way in which the tone is sustained against the depressant influence of the citrate (observation 16, figure 6) is even better evidence than the absolute height of the preliminary peak that the tone-increasing power of the plasma and serum is still in evidence, even in the presence of the citrate, and to an equal degree in both plasma and serum.

It was not considered advisable to test on the uterus and intestine the citrate material of the other experiments which had been used for the ring and perfusion observations simply because it was recognized that the presence of the citrate rendered the liquids less suitable for work with these objects. Only a sufficient number of experiments were made to demonstrate that in the case of the citrate material as well as in the case of the hirudin and peptone material, approximate equality of effect was obtained from plasma and serum. This was considered important since the most striking difference between the plasma and serum in the case of the artery rings and the perfusion preparation had been made out with the citrate material.

### PEPTONE PLASMA AND SERUM.

Material was obtained from two dogs. Material from two other dogs was not used on account of early clotting.

*Experiment of Aug. 22, 1912.*—After about 50 c.c. of blood had been drawn from one carotid artery of a dog, and defibrinated, 0.7 gm. per kilogram of Witte's peptone was injected into the femoral vein. A 10 per cent. solution of the peptone made up in a 0.9 per cent. sodium chloride solution was used. Blood was then drawn in twenty-five minutes through a cannula (boiled and oiled) in the other carotid. The plasma from this blood although it clotted later showed no signs of coagulation before or during the vessel ring experiment. The effect on the ring was fully as great as that of the serum.

*Experiment of Aug. 24, 1912.*—From one carotid of a dog blood was drawn and defibrinated. Then 0.7 gm. of peptone per kilo of body weight was injected intravenously as in the preceding experiment. The injection (58 c.c.) was made in two minutes (2:24 to 2:26 P. M.). Six samples of blood were then drawn off from the other carotid at the following times: (1), 2.29; (2), 2.32; (3), 2.36, (4), 2.51; (5), 3.10; (6), 3.20 P. M.

*Artery Rings.*—The effect of most of the specimens of peptone plasma was as great as that of serum from the same animal. In two observations the peptone plasma produced a somewhat greater effect than the serum. In two observations the action of the plasma was somewhat inferior to that of the serum. Obviously the results of the comparison between peptone plasma and serum are very different from the corresponding results for citrate material. This is well illustrated in the following condensed protocol showing the results of the ring tests with the material of August 24.

In this experiment the carotid of a dog was used for the artery rings, the only occasion on which an artery other than the sheep's carotid was employed. The rings being smaller, the rise observed was not so great. Nevertheless the effects were prompt and well marked. The results of comparing the various plasmas with serum from the same dog may be summarized as follows:

*Plasma 1.*—Not as great as serum.
*Plasma 2.*—About the same as serum.
*Plasma 3.*—Had clotted before the tests were made.
*Plasma 4.*—Fully as great as serum.
*Plasma 5.*—Not quite equal to serum.
*Plasma 6.*—Had clotted before the tests were made.
None of the material used for the ring tests had shown any clotting.

No observations were made with the peptone material on the perfusion preparation.

*Intestine.*—For comparison with the peptone plasma it was necessary to use serum either undiluted or diluted with Ringer to a known extent. Peptone was not added to the serum, as the amount of peptone in any plasma was unknown. Since the plasma was obtained by a reaction within the body,—a veritable intoxication indeed,—exact comparison of plasma and serum was in any case rendered impracticable by the fact that in addition to the substance which restrains clotting other substances formed in the reaction of the organism to peptone may be present in the plasma and may affect the intestine and uterus. Nevertheless the general resemblance between the action of peptone plasma and serum on these test objects is not obscured, as is shown in the tracings reproduced in figures 8 to 11. It is worthy of note that plasma 5 (figure 10. observation 18), which clotted before it was used, instead of causing a larger increase of tone than plasma 1 or 4, produced a considerably smaller increase. Too much stress, however, must not be laid on this, for plasma 2 (figure 10, observation 12), which had not clotted, produced an even smaller effect, and therefore an effect much inferior to that of the other unclotted plasmas. The cause of this difference in the effects was not due to a deterioration of the segment, since after these observations it still gave almost as great an increase of tone with the serum as at first. As already remarked, the changes in the blood by the reaction of the organism to the peptone could not be controlled and these probably influenced the action of the various specimens of plasma in different degrees. The peptone itself, as shown in figure 11, caused some inhibition of the segment, and this might also be a factor in producing the differences between the specimens, although the presence of unchanged proteose cannot itself account for them, since it is to be supposed that more would be found in plasma 1 than in plasma 2.

### HIRUDIN PLASMA AND SERUM.

Experiments were made with material obtained from eight animals.

*Artery Rings.*—Hirudin plasma always gave some effect except with the least sensitive preparations. Occasionally the effect of the plasma was marked. In no instance, however, did the plasma produce as great an effect as the serum, while in general the plasma effect was distinctly inferior (figure 1, observations 1 to 5). Yet on the whole the difference between the hirudin plasma and serum was less than the difference between the citrate plasma and serum. No clotting was ever observed in the plasma during contact with the rings, although carefully looked for. While some of the preparations clotted later, this did not occur for many hours. Most of them did not clot during the period for which they were observed (several days in the ice chest).

*Protocols.*—Aug. 29, 1912. Dog blood was run into 25 c.c. of hirudin solution (1 mg. per c.c.) to make 100 c.c. For the serum 60 c.c. of defibrinated blood was mixed with 20 c.c. of the hirudin solution.

*Results of the Ring Tests.*—Plasma, moderate rise; hirudin serum, good rise; serum from defibrinated blood, good rise.

Sept. 10, 1912. (1) Plasma. Blood was run into 20 c.c. of hirudin solution (1 mg. per c.c.) to make 100 c.c.

(2) Serum. 80 c.c. of defibrinated blood were mixed with 20 c.c. of hirudin solution.

(3) Same as (1); collected after (2).

(4) Same as (2); collected after (3).

(5) Serum from defibrinated blood; collected after (4).

(6) Serum from clot; collected after (5).

Great care was taken in collecting the hirudin blood and no flaw was discoverable in the technique.

*Results of the Ring Tests.*—Plasma, good rise; hirudin serum, very good rise, more than with plasma; hirudin solution, no effect; serum without hirudin, very good rise, about the same as with hirudin serum.

Sept. 13, 1912. Plasma. 40 c.c. of blood from a dog's carotid were run into 20 c.c. of hirudin solution (1 mg. per c.c.).

Hirudin serum. 20 c.c. of hirudin solution were added to 40 c.c. of defibrinated blood.

*Results of the Ring Tests.*—Plasma, slight rise; hirudin serum replacing plasma, very good rise; hirudin replacing Ringer, very good rise. Same material tested on Sept. 14. Hirudin plasma, slight rise; hirudin serum, good rise.

Sept. 26, 1912. Blood was obtained from two rabbits, with the usual precautions. From each rabbit 30 c.c. of blood were run into 10 c.c. of hirudin solution (1 mg. per c.c.). To defibrinated blood from the same animals (a mixture of approximately equal quantities of blood from each rabbit) a corresponding amount of hirudin solution was added. The two specimens of unclotted hirudin blood first obtained were mixed. The preparations were kept in the incubator for about thirty minutes before centrifugalization.

*Results of the Ring Tests.*—Plasma, moderate effect; hirudin serum displacing plasma, good effect; hirudin serum displacing Ringer, very good effect; hirudin solution in Ringer (dilution the same as in the plasma, as indicated by the relative volume of corpuscles and plasma in the blood), no effect.

Oct. 2, 1912. Plasma. 60 c.c. of dog blood were run into 20 c.c. of hirudin solution (1 mg. per c.c.).

Hirudin serum. 60 c.c. of defibrinated blood were mixed with 20 c.c. of the hirudin solution.

*Results of the Ring Tests.*—Plasma, moderate rise; hirudin serum, good rise; plasma (ring stretched in hirudin Ringer solution instead of in Ringer), moderate rise, but no smaller than that given by the plasma replacing Ringer.

On Oct. 3, 1912, the unclotted hirudin blood of Oct. 2 was diluted with such a volume of Ringer as made with the hirudin solution previously added a final dilution of the blood with its own volume of solution. The same was done with the defibrinated blood. The dilute plasma and serum were then separated and tested on artery rings with the following results; serum, good rise; plasma, slight rise; serum displacing plasma, good rise; plasma, slight rise; serum displacing plasma, good rise.

In this experiment the relative activity of serum and plasma in the dilution mentioned resembled more closely the relative effects of citrate plasma and serum, which were always obtained from blood diluted to the same extent.

Since the hirudin plasma and serum obtained in our experiments were less diluted than the citrate plasma and serum it seemed desirable to ascertain whether this factor had any influence in producing the difference noted in the relative effect of the plasma and serum in the two cases. Figures 12 and 13 illustrate observations made for this purpose with the material of October 16. In figure 12 at 1, dog's citrate plasma, of the concentration ordinarily used in our experiments (that is, obtained from blood diluted with its own volume of citrate solution) and referred to in the description of the figure as plasma A, replaced Ringer and was itself replaced at 2 by the corresponding citrate serum (serum A). At 3 citrate plasma (plasma B), from the same animal obtained from blood diluted with half its own volume of a Ringer citrate solution containing twice the concentration of citrate ordinarily used for plasma, replaced Ringer, and was itself replaced at 4 by the corresponding citrate serum (serum B). It will be seen that in the greater concentration the plasma and serum effects are increased in approximately the same proportion. Accordingly, the greater concentration of the hirudin material as ordinarily tested does not of itself account for the difference referred to. Observations 5 and 6, and 7 and 8, were repetitions respectively of observations 1 and 2, and 3 and 4, on rings from the same artery. Although the absolute amount of the rise is different, yet the proportion between the serum and plasma effect is not greatly altered and is still of the same order of magnitude as we have always observed with the citrate material.

On rings of another sheep artery (figure 13) observations 14 and 15, corresponding to 1 and 2, and observations 16 and 17, corresponding to 3 and 4, of figure 12 show even more clearly that so long as the concentration is kept the same in both a mere difference of concentration in the plasma and serum does not conceal the great difference in their pressor action. For comparison observations 18 and 19 (figure 13) are reproduced, showing the effect of the citrate serum in the two dilutions when the Ringer was directly replaced by it. In figure 14 with rings from a third sheep's artery, the effects of serum from the same dog (without citrate), diluted with different amounts of Ringer, are displayed. The details are given in the description of the figure.

*Perfusion Preparation.*—Hirudin plasma and serum differ less in their effect on the rate of perfusion than do citrate plasma and serum. In certain cases the effect of the plasma, although the maximum slowing was as great as that produced by the serum, was less durable. In a number of instances the total plasma effect was fully as great as the serum effect. No indication of clotting was ever seen in the diluted plasma which was injected. Figures 2 and 3 give specimens of the curves showing the changes in outflow in two of the experiments (those of September 26 and October 2). Figure 5 shows a specimen of the drop record from the experiment of September 26.

*Intestine.*—No appreciable difference was found in the effect of hirudin plasma and serum on this preparation. This confirms the conclusion previously reached by one of us.[10] Only two experiments, one on dog blood and the other on human blood, were referred to in that paper, but these were part of a larger series which was continued in the present investigation. It may be well to explain that in these two experiments Ringer's solution without hirudin was added to the defibrinated blood, to give the same dilution as in the case of plasma. This was done with the idea of permitting a more exact appraisal of the advantages of plasma as compared with serum for the detection of adrenalin in the peripheral blood with the test objects (intestine and uterus) previously employed, since in the previous work serum alone had been used. For the purpose of comparing the effects of plasma and serum on the tone of the intestine and uterus segments, the absence of hirudin in the defibrinated blood in these two experiments really rendered the tests more severe, since the hirudin solution itself (in Ringer) caused a slight fall of tone of the intestinal segment, with some diminution in the size of the beats. In all the other experiments hirudin was added to the defibrinated blood in the same proportion as to the whole blood. The results were identical. In no instance was there any distinct and constant preponderance of effect on the part of the serum.

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[10] Stewart, G. N., *Jour. Exper. Med.*, 1912, xvi, 502.

That this was not due to the effect of the serum being a maximal response on the part of the preparation was shown (1) by obtaining markedly greater effects on the same preparation with undiluted serum, and (2) by comparing the effects of serum diluted sufficiently to cause a response much below the maximal with that of plasma diluted to the same degree. The effects were practically identical with the diluted plasma and serum (figures 16 and 17).

A fact of prime importance to be borne in mind in interpreting the tracings from the intestine preparation, and one that was noted in previous papers, is that not infrequently the first observation, or occasionally the first two or three observations, show a much smaller response, at any rate as regards increase of tone, than subsequent observations. This is especially the case when the segment is beating feebly in Ringer solution. It then seems to require some time when the Ringer is replaced by serum or plasma to attain the nutritive condition in which it responds readily to the tone-increasing property of the serum or plasma, the first effect of the serous liquid being to increase the size of the beats. After this stage it will go on responding for a long time through many successive observations with remarkable steadiness. If now with such a preparation a comparison of plasma and serum were begun by the addition of plasma (as in observation 2, figure 17) the change of tone would appear far inferior to that occasioned by a subsequent application of serum, and if the experiment stopped here (before observation 6, for example, had been made in figure 17), the same relation between hirudin plasma and serum as regards their effect on the intestine might be supposed to be demonstrated which undoubtedly exists for citrate plasma and serum in the case of artery rings. Yet this impression would be at once corrected by performing the observations in the reverse order (as in observation 33, figure 18, or observation 2, figure 19). The serum would now appear to increase the tone of the segment far less than the plasma, an equally erroneous conclusion, as shown by observation 37, figure 18, with the same serum.

*Uterus.*—For the uterus the same general statement may be made as for the intestine in regard to the action of the hirudin material. The parallelism is even more impressive in the case of the uterus because it exists not only where the uterus responds to serum by an immediate increase of tone, but also where the primary effect of the serum is an inhibition. Thus in figure 20 the effect of the hirudin plasma from rabbits of September 26 on rabbit uterus (observation 39) is to cause practically the same immediate increase of tone as the corresponding hirudin serum (observation 41). That this is not because both effects are maximal is shown by observations 47 and 49 in which the hirudin plasma and serum were each diluted with five volumes of Ringer. The increase of tone produced by the dilute plasma is here, indeed, greater and somewhat more durable than that produced by the dilute serum. In figure 21 dog hirudin plasma (observation 28) and hirudin serum (observation 26) of September 12 caused practically identical and very prompt increases of tone in a rabbit uterus segment, the increase in each case being somewhat greater than that occasioned by the serum of the same dog without hirudin but diluted with its own volume of Ringer.

In figures 22 and 23, on the other hand, material obtained from a dog on August 29 caused in a rabbit uterus preparation as its first effect a marked relaxation. Observations 24 and 28 (figure 22) are with hirudin plasma, and observation 26 is an intermediate observation with the hirudin serum. The general similarity between the plasma and serum effects is well marked. Observation 30 (figure 23) shows the effect of the hirudin solution in Ringer, observation 32 that of serum from the defibrinated blood without hirudin, observation 36, the effect of adrenalin 1 : 1,000,000 in Ringer, and observation 42 that of adrenalin 1 : 100,000 in Ringer. The adrenalin observations lend no support to the idea that the inhibition might be an effect of epinephrin in the blood. The inhibitory effect of the serum on this uterus will obviously be intensified by that of the hirudin in the hirudin plasma and serum. The uterus was from an adult non-pregnant rabbit, as was also the uterus used for figures 20 and 21.

### TRANSUDATES AND EXUDATES.

The results of these observations are in harmony with those on serum and plasma. Hydrocele and ascitic fluids, presenting the classical characters of non-coagulable transudates, produced no effect on artery rings. Only the hydrocele fluid was tested on the perfusion preparation. It caused merely a slight slowing in the flow, far less than that caused by the serum used for comparison. On the other hand, its action on the intestine closely resembled that of the blood serum and was at least as great as that of the serum from a typical inflammatory exudate (pleural liquid) which had clotted into a jelly before it was used. For further details and for the action of transudates on the uterus preparation the following paper may be consulted.[11]

### SERUM FRACTIONS AND MODIFICATIONS OF SERUM.

All that need be said at present about these observations is that it proved possible to obtain (1) alcoholic extracts from serum which had no effect upon artery rings and yet caused in the intestine and uterus segments qualitatively the same changes as the original serum (figures 24 and 25), and sometimes quantitatively even greater effects, and (2) modifications of serum (heat preparations) which while differing markedly in their quantitative effect on the intestine and uterus preparations exerted on the artery rings approximately the same effect (figures 24 and 25). This supports the conclusion already drawn from the comparison of serum and plasma that one and the same material may affect the various test objects very differently, and shows that the existence of a marked difference in the action of a given plasma and serum on the artery rings, for example, does not render it *a priori* probable that a similar difference will be found in their action on intestine segments. Further illustrations of this are found in some observations on the action of certain foreign proteins on some of the test objects.

*Protocols.*—Alcohol extracts. July 19, 1912. 100 c.c. of sheep serum were mixed with 400 c.c. of 95 per cent. alcohol. The mixture was stirred occasionally for five minutes and then filtered with suction. The filtrate was evaporated on a boiling water bath.[12] The residue was taken up with 100 c.c. of Ringer's solution and labelled A(1).

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[11] Stewart, G. N., and Zucker, T. F., *loc. cit.*

After precipitating another portion of the serum, filtering, and evaporating as in the preparation of A(1), the residue was taken up with successive portions of absolute alcohol. After filtering, this alcoholic extract was evaporated and the residue taken up with a volume of Ringer solution equal to the original volume of the serum (A(2)).

July 25, 1912. An extract of another sample of sheep serum was made in the same way as A(2) of July 19. It was marked A(3).

*Results of the Ring Tests.*—July 23. A(1), no rise; serum, good rise, A(2), no rise; serum, good rise.

July 25. A(1), no rise; serum, moderate rise; serum diluted with four volumes of Ringer, moderate rise.

July 26. A(3), no rise; serum, very good rise.

*Intestine and Uterus Tests.*—A(1), A(2), and A(3) all produced a good increase of tone, whether tested at once or after being kept for several days. The effect on the intestine, however, was usually less, and was always less durable than that caused by the original serum (figure 24, observation 4), while on the uterus the effect might be decidedly greater than that of the serum (for example the effect of A(3) in observation 16, figure 25). In all the observations in which it was used A(3) gave with the uterus an effect greater than that of the serum, and this is seen in experiments on the uteri of two rabbits separated by an interval of two days (July 25 and 27).

*Heat Preparations.*—July 19, 1912. 100 c.c. of sheep serum were heated in a boiling water bath. After coagulation, 100 c.c. of Ringer solution were added, and the mixture was rubbed up and strained through cloth. The filtrate was labelled H(3).

July 25, 1912. To 50 c.c. of sheep serum obtained on July 25, 200 c.c. of Ringer solution were added and the mixture was heated on wire gauze to boiling. Boiling was kept up for a few minutes. The liquid was then strained through cloth but hardly any coagulum had formed and it passed through as an opalescent liquid. The filtrate was labelled H(5).

Fifty c.c. of the same serum were coagulated in a boiling water bath. The coagulum was rubbed up in a mortar with 200 c.c. of Ringer solution. The solid particles were strained out and the liquid was filtered. The filtrate was labelled H(6).[13]

*Results of the Ring Tests.*—July 24, 1912. H(3) gave a good rise, about the same as that given by the serum used for comparison.

July 26. H(5), good rise; serum diluted with four volumes of Ringer, not as good a rise as with H(5), but the difference is slight; H(6), about the same as serum diluted with four volumes of Ringer.

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[12] Alcohol extracts evaporated at lower temperatures and reduced pressure have a good pressor effect on artery rings.

[13] This protocol is not quoted as a method by which one may obtain with certainty from any serum preparations differing in their action as H(5) and H(6) did. Our point is that these products, however obtained, did in fact show a very different action on the various test objects.

*Intestine.*—H(3) gave a very good and sustained increase of tone quite comparable to that occasioned by the corresponding serum. H(5) (figure 24, observation 15) caused a large increase of tone greater than that produced by the unheated serum similarly diluted with Ringer (observation 9). The contractions disappeared and the increase of tone was soon succeeded by a great fall. Not only quantitatively but qualitatively the effect was quite different from that of the unaltered serum and from that of H(6). This was verified by repeated observations on intestine segments from two rabbits.

H(6) (figure 24, observation 11) produced a rise in the curve somewhat inferior to that produced by the unheated serum similarly diluted. The beats continued just as in the unaltered serum and the increase of tone was well sustained. This similarity in the action of H(6) with that of serum was also verified by a number of observations on preparations from two rabbits. The difference between H(6) and H(5) is most strikingly shown in observations 17 to 20, figure 24, where the replacement by H(6) of serum diluted with four volumes of Ringer produced little effect, while the subsequent replacement of H(6) by H(5) caused the characteristic diminution of tone after a preliminary rise.

*Uterus.*—H(3) affected the uterus preparation much as the corresponding serum did, causing the usual increase of tone. H(5) produced a decidedly greater and more sustained increase of tone than either H(6) or the unaltered serum correspondingly diluted.

## FOREIGN PROTEINS.

As already indicated, the main point of interest in these observations as regards our present subject is the further and, indeed, striking evidence they afford that a material which exerts little or no action on one of the test objects (artery rings) in comparison with serum may have a very decided effect, quite comparable in magnitude with that of the same serum on another test object (intestine segment).

*Protocols.*—Sept. 28, 1912. Egg white. The white of an egg which was laid the same day and in which decomposition products are therefore certainly excluded was strained through several layers of cheese cloth and various dilutions of it with Ringer solution were made.

*Witte's Peptone.*—One gm. of Witte's peptone was rubbed up with 20 c.c. of Ringer solution. A slight sediment remained behind on decanting. Further dilutions were made with Ringer solution.

*Wheat Flour Suspension.*—Schultz states that an extract of the wheat proteins gives a better effect on the guinea pig intestine, but as he states that he also obtains a good effect with a suspension of wheat flour in distilled water we used the latter. This suspension was made by shaking up 25 gm. of wheat flour with 50 c.c. of distilled water. Further dilutions were made with Ringer solution.

*Results of the Ring Tests.*—Egg white, undiluted, slight effect; diluted with one volume of Ringer, very slight effect (figure 1, observation 14); rabbit serum diluted with one volume of Ringer, very good effect (figure 1, observation 16). Dog serum (of Sept. 24) also gave a very good effect. These observations should be compared with the corresponding observations on the intestine preparation (figure 26). It will be seen that the tone-increasing power of the egg white diluted with its own volume of Ringer on the intestine preparation is of the same order of magnitude as that of the rabbit serum correspondingly diluted.

*Witte's Peptone.*—Both the 5 per cent. solution and a 0.5 per cent. solution were practically without effect on the artery rings.

*Wheat Flour Suspension.*—50 per cent. suspension; slight effect. Suspension diluted with 9 volumes of Ringer; very slight effect. On the intestine preparation, the 50 per cent. suspension produced a fair increase of tone, which, however, was transient.

*Intestine.*—Solutions of egg white which had no effect on the artery rings caused a decided increase of tone on the intestine preparation, an increase of tone comparable with that produced by serum (observations 27 and 29, 31 and 33, figure 26).

Solutions of Witte's peptone caused some inhibition of the intestine, which gave place to a remarkably abrupt increase of tone on washing with Ringer's solution (observations 31 and 35, figure 11). No such phenomenon was observed with the artery rings. It does not seem to depend on a difference between the effects of concentrated and dilute solutions of the peptone, as we at first thought might be the case from the statement of Schultz[14] as to the stimulating action of peptone on guinea pig intestine. A possible explanation, but one which we have not tested, is that Witte's peptone contains two substances which act on the intestine, one inhibitory and the other stimulating. The first substance (or its action) is easily removed by washing with Ringer, while the second substance (or its action) is not so easily removed by washing.

Extracts of wheat flour which caused some increase of tone in the intestine preparation were inactive as regards artery rings.

A word or two may be added on the bearing of our results on the technique of epinephrin tests. We believe that Janeway and Park[15] are correct in considering the artery ring method a good one for this purpose, although we are not impressed with the sensitiveness of the ring preparations for adrenalin. Our experience, like that of most previous observers, is that artery rings are inferior in this respect to preparations such as intestine and uterus segments. The great advantage of the artery ring in our opinion is the rela-

[14] Schultz, W. H., *Bull. Hyg. Lab., U. S. P. H. and M.-H. S.*, 1912, No. 80, 37.

[15] Janeway, T. C., and Park, E. A., *Jour. Exper. Med.*. 1912, xvi, 541.

tive constancy of the response in a qualitative sense and its definiteness. The response to epinephrin as well as to serum is, from the nature of the biological mechanism involved, an exceedingly simple, and therefore unambiguous one. The intestine or uterus segment has several ways in which its activity may be altered. The beats may increase or diminish in strength or in rate, and the tone may increase or diminish. The response to adrenalin or serum in one condition of a segment may be different from the response in another nutritive condition. Another advantage of the rings is that the complicating effect of the pressor action of serum can, as a rule, be avoided to a considerable extent with the artery preparation by using citrate plasma. This as we have shown is not the case for intestine and uterus segments.

The use of plasma, however, even for the rings has its disadvantages, of which the gravest is that we cannot be quite certain, even with citrate plasma, that a given pressor effect is due to epinephrin and not to a development, greater than the ordinary for this form of plasma, of the pressor property of shed blood. This difficulty becomes greater with the Laewen preparation even when citrate plasma is used, and still greater, both with the rings and the perfusion preparation, when hirudin plasma is employed. We are therefore impressed by the argument of Kahn in which he deprecates the abandonment of serum for the perfusion method in favor of plasma, as recommended by O'Connor, and points out that, although the constrictor effect of a serum may often be greater than that of the corresponding plasma, it is much more constant and can accordingly be more easily taken account of. For the intestine and uterus preparations, as we have shown, plasma offers no advantages over serum. Anyone who desires to work with these preparations on account of their superiority in sensitiveness to the other preparations will therefore naturally employ serum. Hence we fail to see any reason for the statement of Janeway and Park that "Stewart's negative conclusions are invalidated by the fact that he worked with serum." The further statement of these authors, that "Stewart obtained epinephrin reactions constantly only when the adrenal gland was handled roughly or massaged, and regularly failed to obtain such reactions when precautions against this were taken even though the splanchnics were stimulated," is quite erroneous and doubtless based on some misapprehension.

### DISCUSSION AND SUMMARY.

Apparently, then, we are confronted with this result,—that citrate plasma which causes little or no constriction of the stretched artery ring, little or no slowing of the flow through the frog perfusion preparation, while the corresponding serum produces a marked effect on both preparations, will affect the intestine or uterus preparation practically in the same way as the corresponding serum. Hirudin plasma and serum exert on the intestine and uterus preparations practically the same effect, causing a marked increase of tone. On the artery ring preparation, there is a difference although it is not so strongly marked as in the case of the citrate material. The frog perfusion preparation, as regards the effect of the hirudin material, seems to occupy an intermediate position between the intestine and uterus on the one hand, and the artery ring on the other.

Of the three plasmas, the peptone plasma most closely resembles serum in its action on the artery rings. Like the other plasmas its effect on the intestine and uterus does not differ appreciably from that of the serum. From these observations the following conclusions seem justified.

A change occurs in shed blood which confers on it the property of constricting artery rings and of slowing the flow through the perfused frog preparation. If this property is in any degree possessed by circulating blood it is at least markedly increased after the blood is shed. The change, whatever it may be, does not entail any essential alteration in the action of the blood on intestine and uterus segments. The tone-increasing property developed in the shed blood may, therefore, so far as the four test objects included in the present survey are concerned, be looked upon as especially affecting the blood vessels and probably their smooth muscle directly. This need not imply that the pressor substance, if it is a single definite substance developed in the shed blood, exerts no action on the smooth muscle of the intestine and uterus preparations, but merely that its action on these objects is masked by the general action of the serum and plasma, so that in the presence of the other constituents common to serum and plasma, its effect is inconspicuous or not to be detected at all, while on the blood vessel preparations, especially the artery rings, the effect of the pressor substance is the dominant one, and the general action of the serum and plasma is feeble or undetectable.

It is not the clotting process as such, *i. e.*, the actual change of fibrinogen into fibrin, that is responsible for the differences between serum and plasma revealed by the biological tests employed. but some process which precedes or accompanies the clotting and which may or may not be causally related to it. Changes in formed elements of the blood under the influence of the changed conditions (contact with foreign bodies, restriction of gaseous exchange, etc.), which blood encounters as soon as it leaves the living vessels, are known to occur. Among these it is to be assumed are the changes which condition the differences between plasma and serum under discussion.[16] Even if these changes represent preliminary stages in coagulation (liberation of the factors necessary to the formation of thrombin, for instance), they may still occur to a greater or less extent in blood which is prevented in certain ways from clotting, since it is known that the procedures by which the various non-coagulable plasmas are obtained break at different points the chain of events which normally ends in coagulation. A procedure which simply supplies sufficient antithrombin to neutralize the thrombin which has been allowed to form in normal amount may not interfere at all with the changes in the formed elements, and the pressor property of the resulting plasma may then be as marked as that of the serum. On the other hand, a procedure which hinders clotting by preventing or diminishing the alterations in the cells, for example, the addition of a substance which acts as a preservative for blood platelets, will very likely yield a plasma with little or no pressor effect in comparison with the serum. The mere prevention of clotting, then, except in so far as it is an index of the prevention of changes in the formed elements may have little significance in preventing the development of the pressor property. Indeed it is

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[16] Since this was written we have found that extracts of platelets obtained from citrate blood, the plasma of which is totally inactive, exert a strong constricting effect on artery rings.

## EXPLANATION OF PLATES.

All tracings are to be read from left to right. Time is marked in half minutes. Unless otherwise mentioned the liquid to be tested was always caused to displace the Ringer solution in which the preparation was immersed. The original relation both to base line and to time trace is preserved in figures 6 to 10, 14, 15, and 19. The relative distance from the base line is preserved in figures 16 to 18, 20 to 23, and 26, but all the curves are brought up closer to the time trace.

### PLATE 11.

FIG. 1. Sheep artery rings. For observations 1 to 5 the rabbit hirudin material of Sept. 26 was used; for observations 6 and 7 the dog citrate material of Sept. 24. In observations 14 and 16 a dilution of egg white was compared with a dilution of serum. The arteries were from different animals in the three sets of observations. Of the two time traces in the lower right hand portion of the figure the upper belongs to observation 3, and the lower to observations 6 and 7. The time trace in the lower left hand portion of the figure belongs to observations 4 and 5. The time trace at the top of the figure corresponds to all the other observations. At 1 hirudin plasma replaced Ringer and was itself replaced at 2 by the corresponding hirudin serum. At 3 Ringer was replaced directly by the hirudin serum. At 4 Ringer was replaced by the hirudin solution in Ringer and this at 5 by the hirudin plasma. At 6 Ringer was replaced by citrate plasma and this at 7 by the corresponding citrate serum. At 14 egg white diluted with one volume of Ringer replaced Ringer. At 16 Ringer was replaced by rabbit serum of Sept. 28.

FIG. 2. Curves showing the flow through the frog perfusion preparation. The number of drops per half minute is laid off along the vertical axis and the time in half minutes along the horizontal axis, each small division representing a half minute. Thirty-eight drops correspond to 1 c.c. The upper set of curves was obtained with dog citrate material of Sept. 24, the lower set with the rabbit hirudin material of Sept. 26. The small rise which sometimes occurs immediately after the injection is due to increase of pressure by the injection.

FIG. 3. Curves showing flow through frog perfusion preparation. Hirudin material of Oct. 2. For the upper left hand curve the pressure was 16 cm. of the perfusion fluid; for the others 21 cm.

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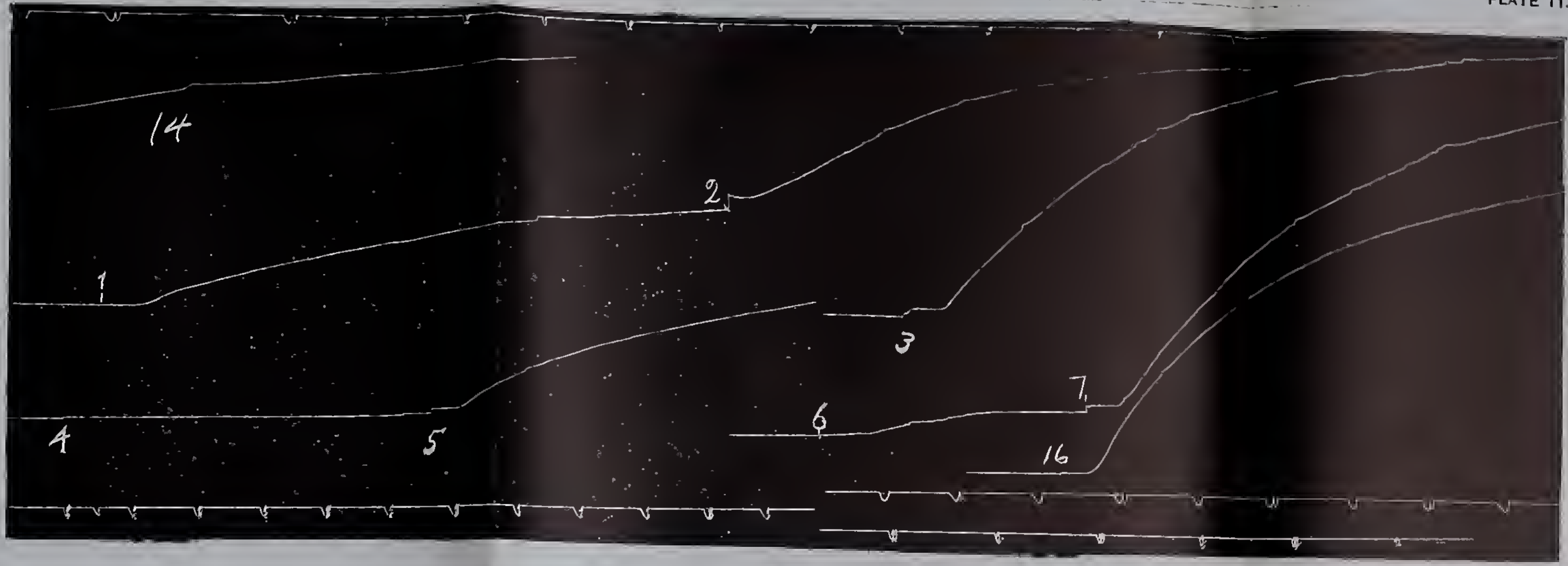

FIG. 1.

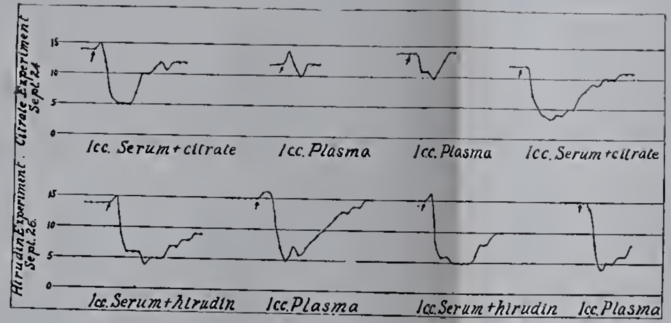

FIG. 2.

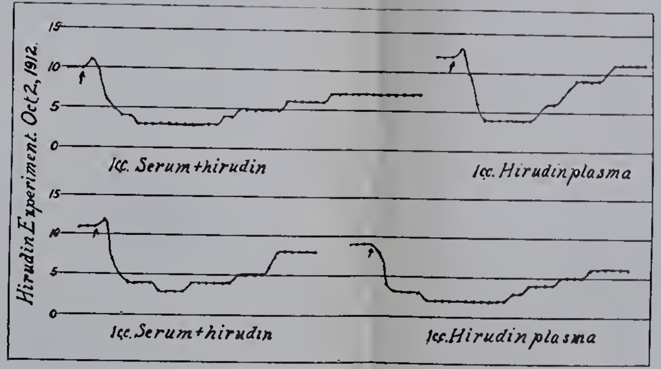

FIG. 3.

(Stewart and Zucker: Comparison of Action of Plasma and Serum.)

### Plate 12.

Fig. 4. Tracings showing the change in the rate of drops from the frog perfusion preparation. At 11 citrate plasma of Sept. 24, at 13 the corresponding citrate serum was injected, in each case 1 c.c.

Fig. 5. Tracings showing the change in rate of drops from the frog perfusion preparation. At 12 rabbit hirudin plasma of Sept. 26, at 13 the corresponding hirudin serum was injected, in each case 1 c.c.

Fig. 6. Intestine. At 4 citrate plasma of Sept. 24, at 6 the citrate serum, at 16 the citrate Ringer solution diluted with its own volume of Ringer replaced the Ringer solution. The magnification of the contraction was about one half of that usually employed.

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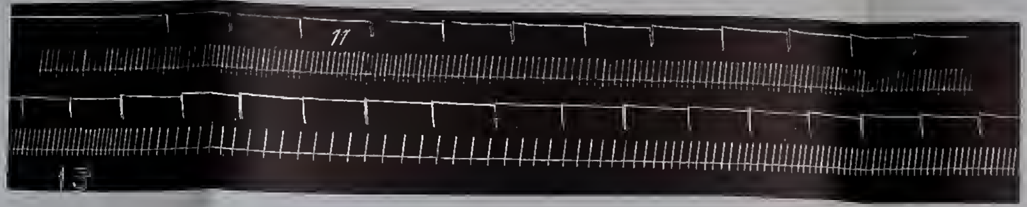

Fig. 4.

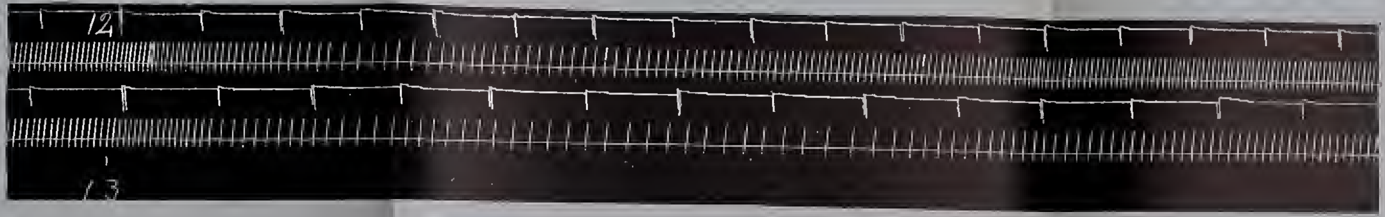

Fig. 5.

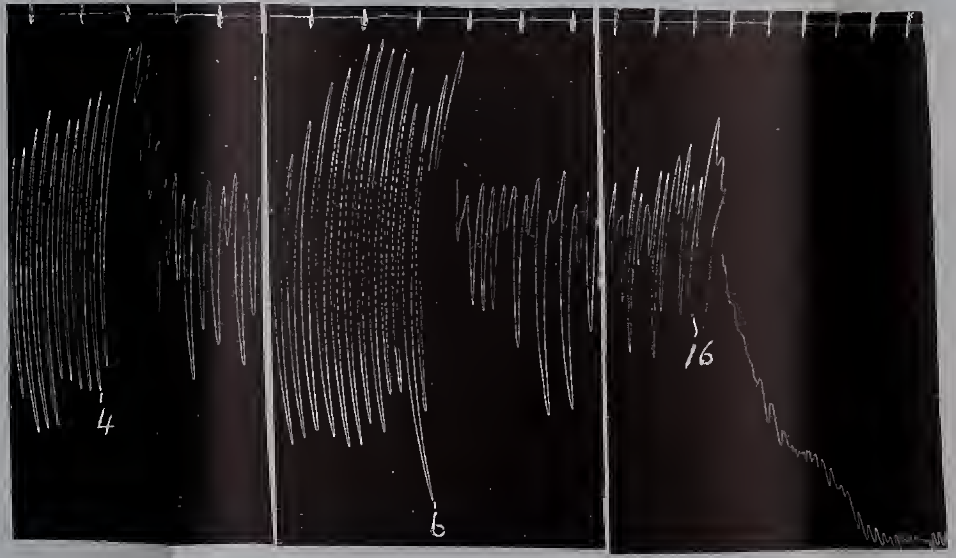

Fig. 6.

(Stewart and Zucker: Comparison of Action of Plasma and Serum.)

## PLATE 13.

*Fig. 7.* Uterus. (From an adult non-pregnant rabbit.) Citrate material of Sept. 24. At 2 serum from clot, at 4 citrate plasma, at 6 citrate serum, at 8 citrate plasma replaced Ringer. All the material was from the same dog. At 11 Ringer was replaced by citrate plasma, at 12 additional citrate plasma was added to replace that removed by the oxygen stream. At 13 the citrate plasma was replaced by citrate serum.

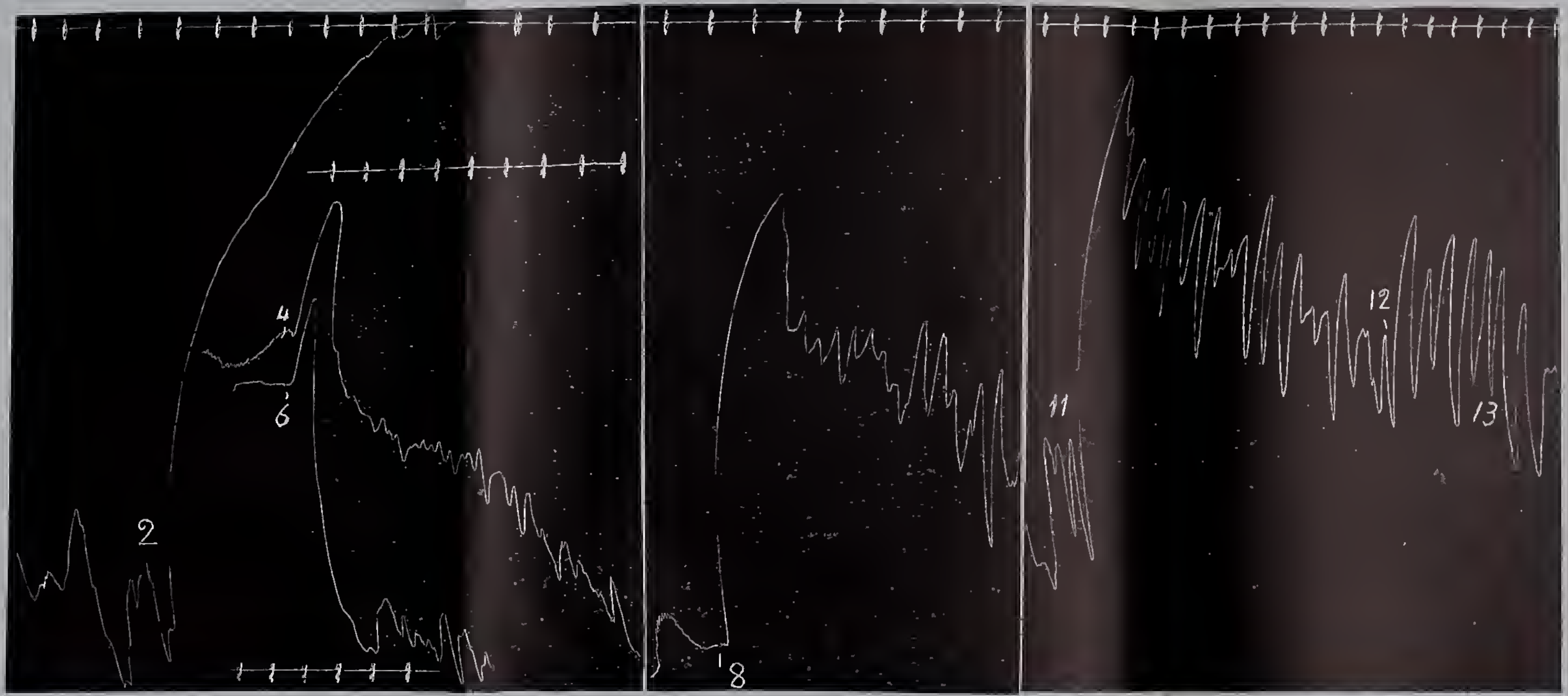

FIG. 7.

(Stewart and Zucker: Comparison of Action of Plasma and Serum.)

## PLATE 14.

FIG. 8. Intestine. At 2 serum, at 4 peptone plasma 1 of Aug. 24 replaced Ringer.

FIG. 9. The same preparation as used for figure 8. At 6 serum, at 8 peptone plasma 1 replaced Ringer when the initial tone of the segment was considerably higher than in figure 8.

FIG. 10. The same intestine preparation as in figures 8 and 9. At 10 peptone plasma 4 and at 14 serum replaced Ringer. At 12 peptone plasma 2, at 18 peptone plasma 5 replaced Ringer. Plasmas 1, 2, and 4 showed no clotting whatever. Plasma 5 was visibly clotted long before the observation was made.

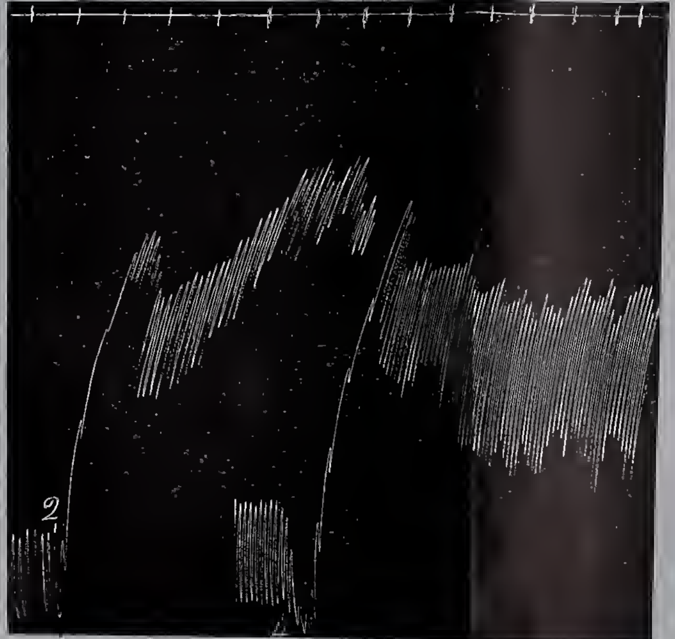

FIG. 8.

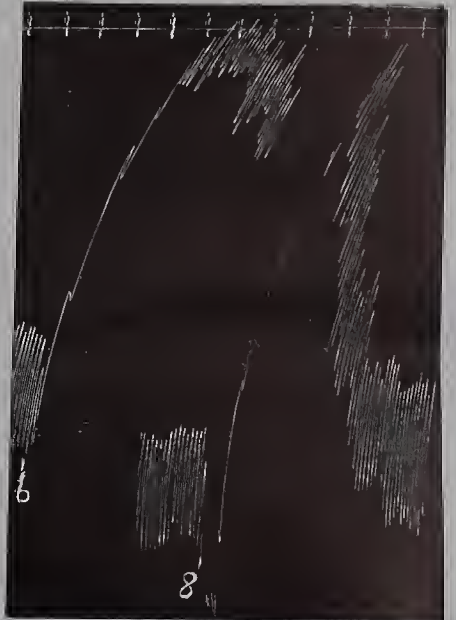

FIG. 9.

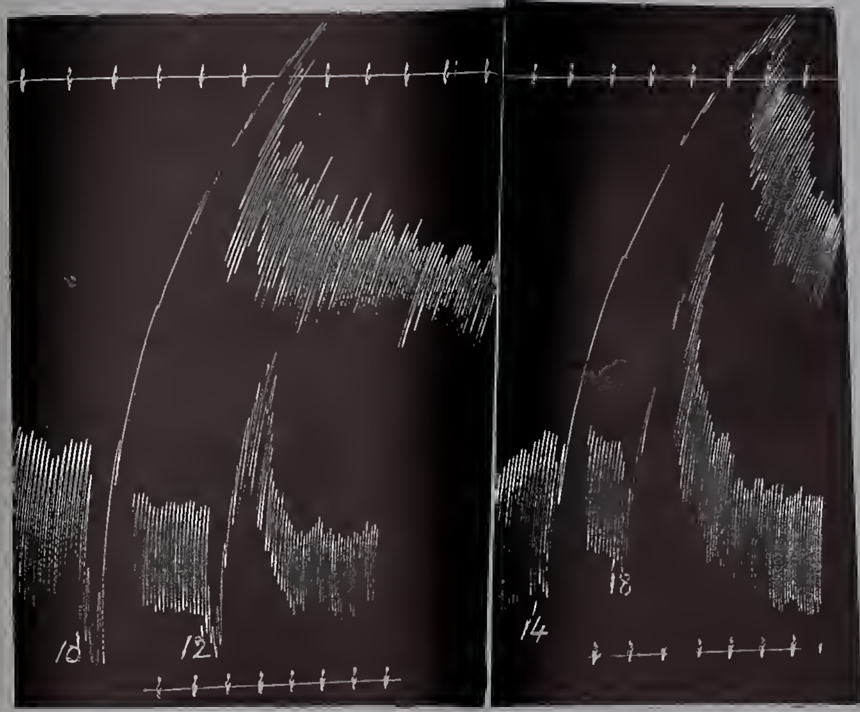

FIG. 10.

(Stewart and Zucker: Comparison of Action of Plasma and Serum.)

## PLATE 15.

FIG. 11. The same intestine preparation as in figures 8 to 10. At 22 a 1 per cent. solution of peptone in Ringer replaced Ringer. At 30 a 2 per cent. peptone solution in Ringer replaced Ringer. At 31 washing with Ringer was commenced, at 32 suspended, at 33 resumed. At 34 a 10 per cent. peptone solution in Ringer replaced Ringer. At 35 washing with Ringer was begun, at 35a suspended, at 36 resumed, and again commenced at 37.

FIG. 12. Sheep artery rings. Citrate material of Oct. 16 (page 162.) At 1 plasma A replaced Ringer and was itself replaced at 2 by serum A. At 3 plasma B replaced Ringer and was itself replaced at 4 by serum B. 5 and 6 are repetitions of 1 and 2 on another ring from the same artery. 7 and 8 are repetitions of 3 and 4 on another ring from the same artery. The immediate small rise at 8 was due to accidental interference with the thread.

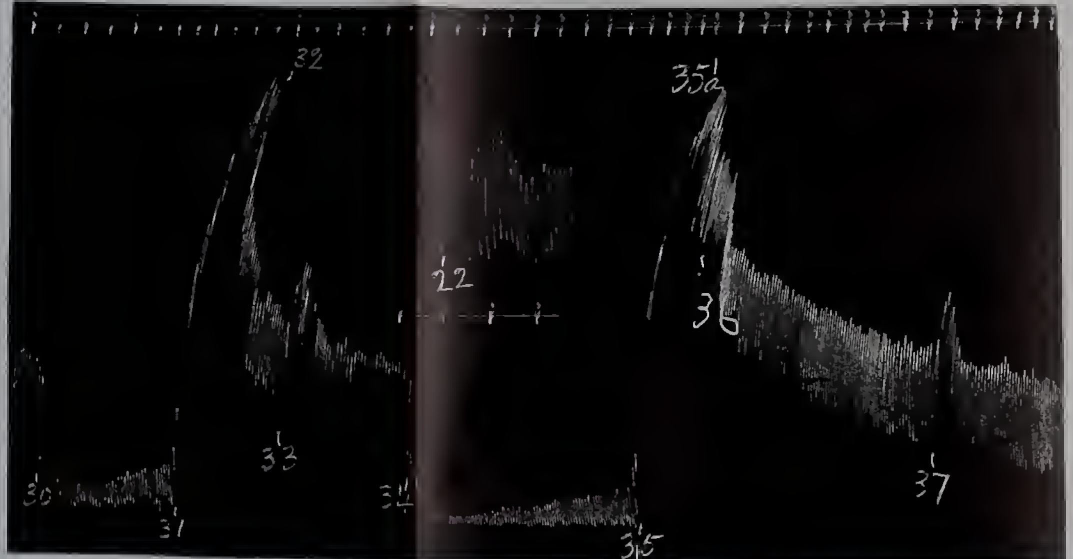

FIG. 11.

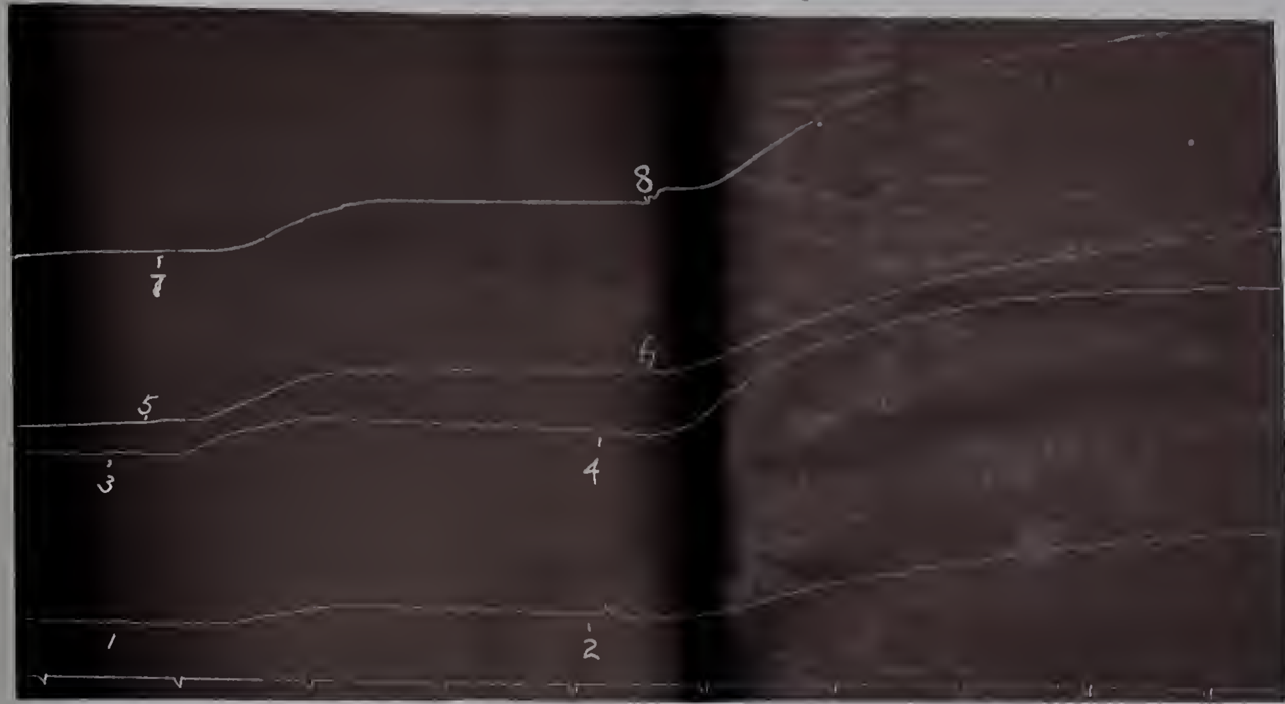

FIG. 12.

(Stewart and Zucker: Comparison of Action of Plasma and Serum.)

## Plate 16.

Fig. 13. Sheep artery rings. Citrate material of Oct. 16. Observations 14 to 17 correspond to observations 1 to 4 in figure 12, but were done with rings from another sheep's artery. At 18 serum A replaced Ringer. At 19 serum B replaced Ringer. The upper time trace belongs to observations 16 and 17, the lower to observations 14, 15, 18, and 19.

Fig. 14. Sheep artery rings. Dog serum of Oct. 16 without citrate. At 9 Ringer was replaced by undiluted serum, at 10 by serum diluted with 1 volume of Ringer, at 11 by serum diluted with 2 volumes of Ringer, at 12 by serum diluted with 8 volumes of Ringer. A repetition of observation 12 on another ring gave precisely the same result.

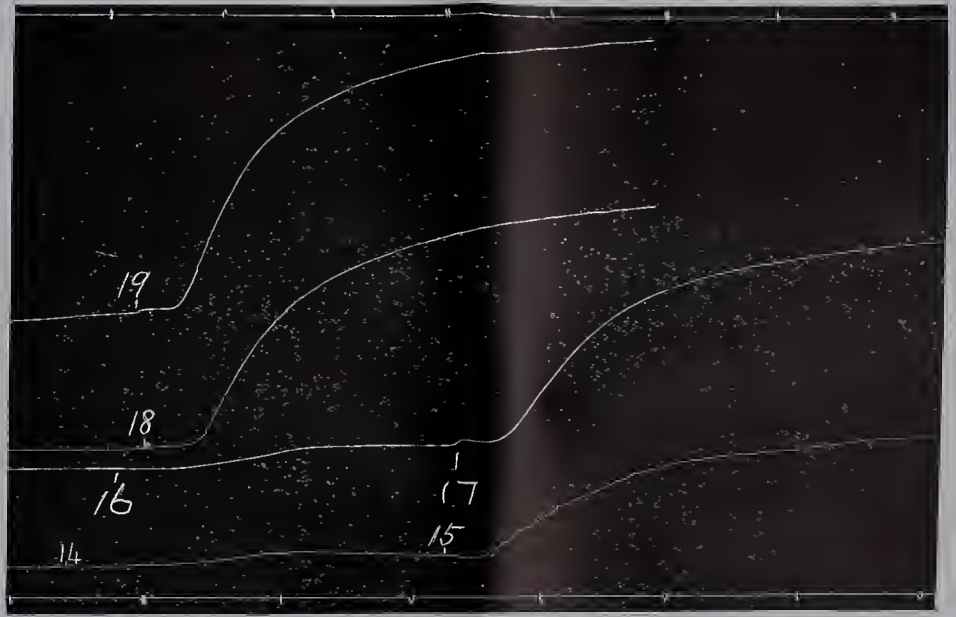

Fig. 13.

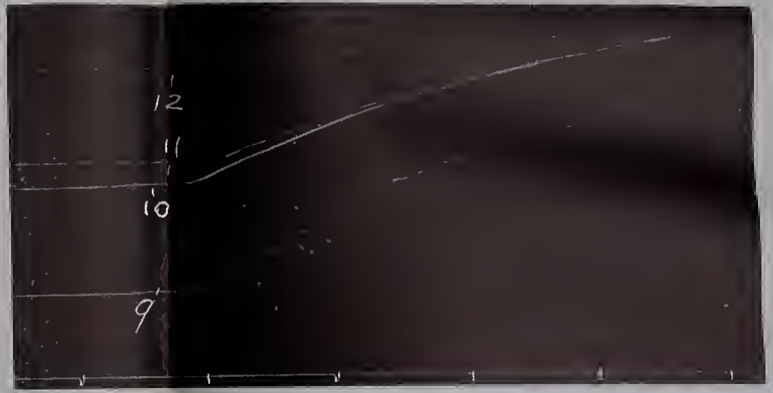

Fig. 14.

(Stewart and Zucker: Comparison of Action of Plasma and Serum.)

PLATE 17.

FIG. 15. Intestine. Rabbit hirudin material of Sept. 26. At 2 hirudin
rum, at 4 hirudin plasma, at 6 hirudin serum replaced Ringer. At 10 hirudin
rum diluted with two volumes of Ringer, at 12 hirudin serum diluted with
e volumes of Ringer, at 14 hirudin plasma diluted with two volumes of
nger, and at 16 hirudin plasma diluted with five volumes of Ringer replaced
nger. Weight of intestine preparation, 0.37 gm.

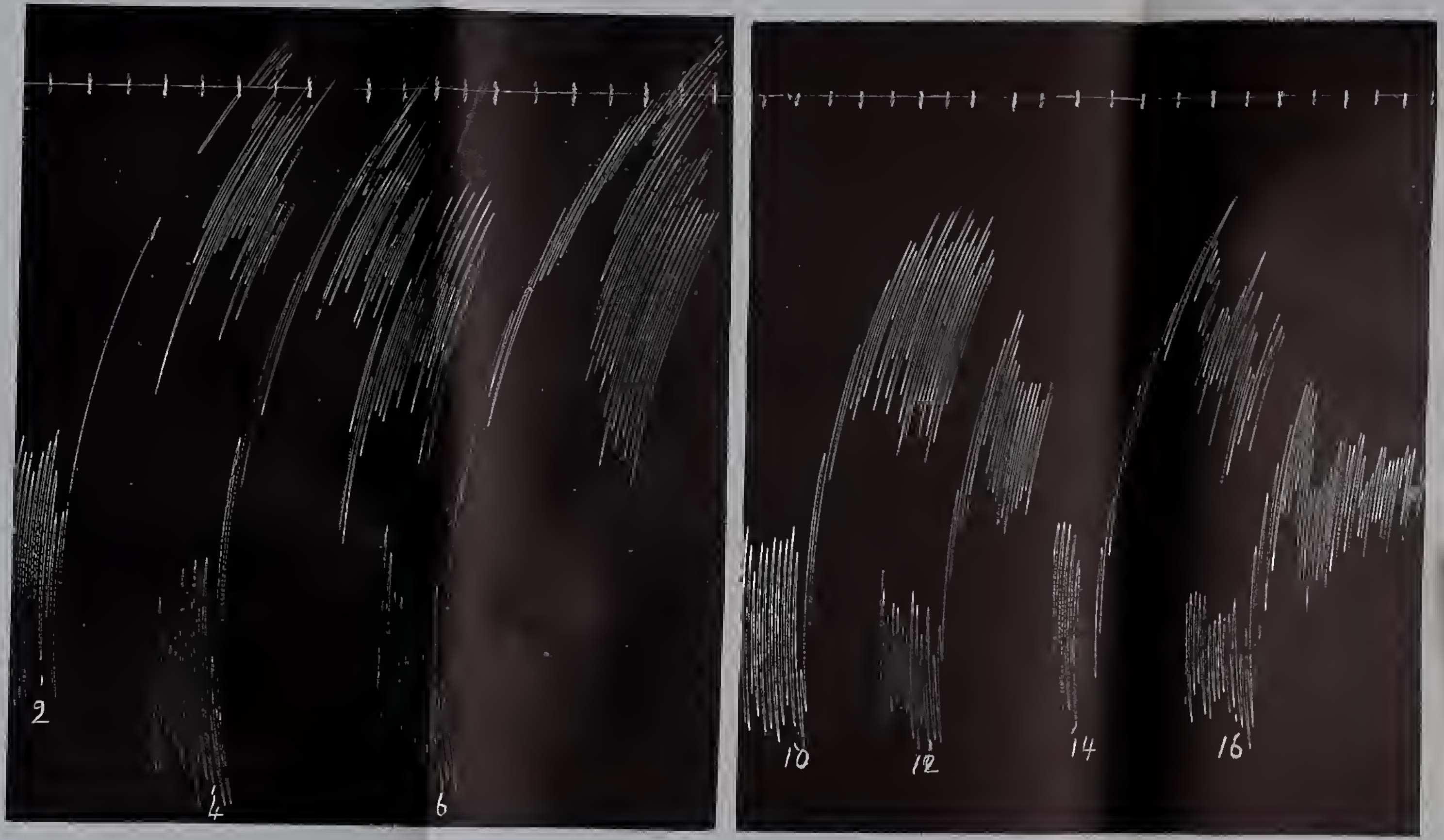

FIG. 15.

PLATE 18.

Fig. 16. Intestine. Hirudin material of Sept. 12. At 6 hirudin serum, and at 8 hirudin plasma replaced Ringer. At 10 Ringer was replaced by serum from the dog's clot diluted with its own volume of Ringer. The magnification of the movement by the lever was 2½ times less than that usually employed. Weight of intestine preparation, 0.40 gm.

Fig. 17. Intestine. Hirudin material of Aug. 29. At 2 hirudin plasma, at 4 hirudin serum, and at 6 hirudin plasma replaced Ringer. The segment was freshly prepared and observation 2 was the first one made on it. Weight of intestine preparation, 0.15 gm. It was much shorter than usual.

Fig. 18. Intestine. Rabbit hirudin material of Sept. 26. At 33 hirudin serum, at 35 the corresponding plasma, and at 37 the hirudin serum replaced Ringer. The intestinal segment was freshly prepared, and observation 33 was the first one made on it. Its weight was 0.34 gm.

Fig. 19. Intestine. Hirudin material of Oct. 2. At 2 hirudin serum, at 4 hirudin plasma, at 7 hirudin plasma, and at 12 hirudin serum replaced Ringer. Observation 2 was the first observation on this segment.

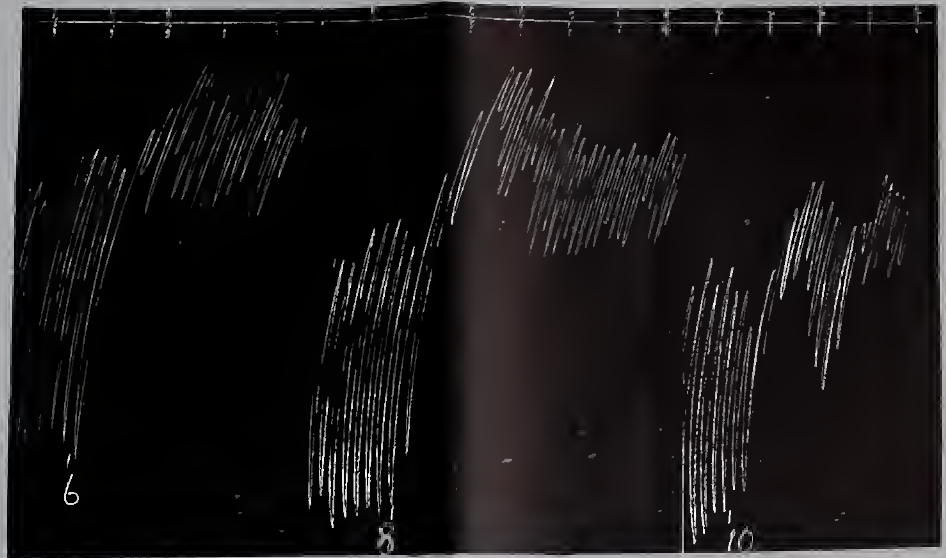

Fig. 16.

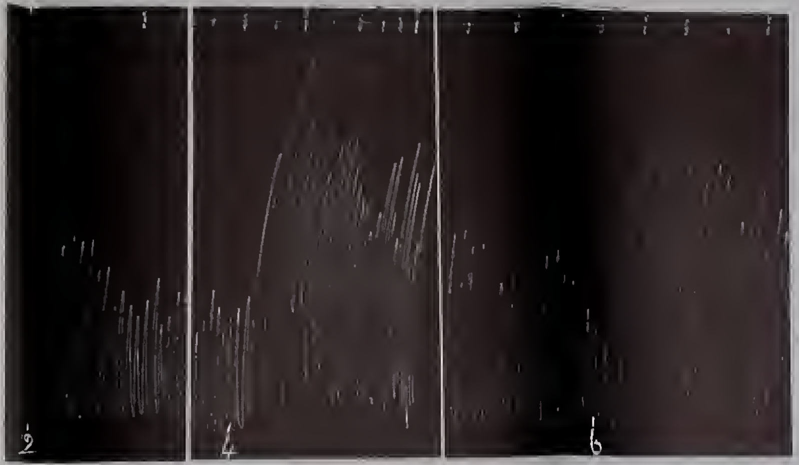

Fig. 17.

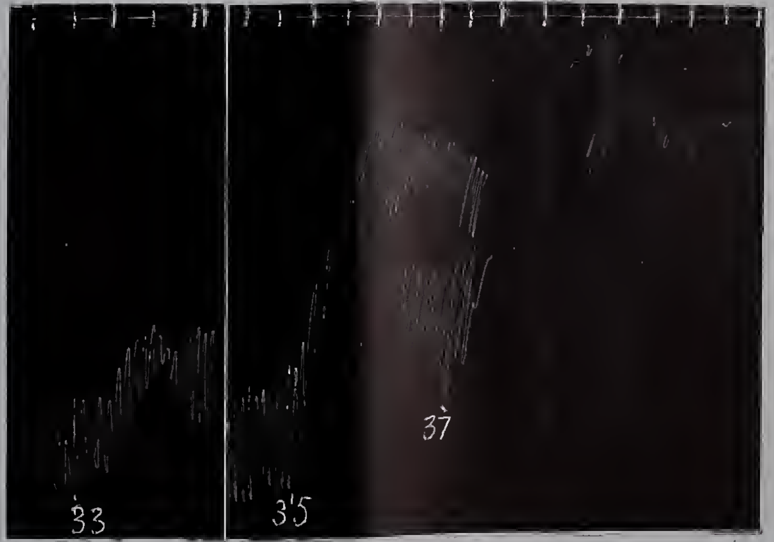

Fig. 18.

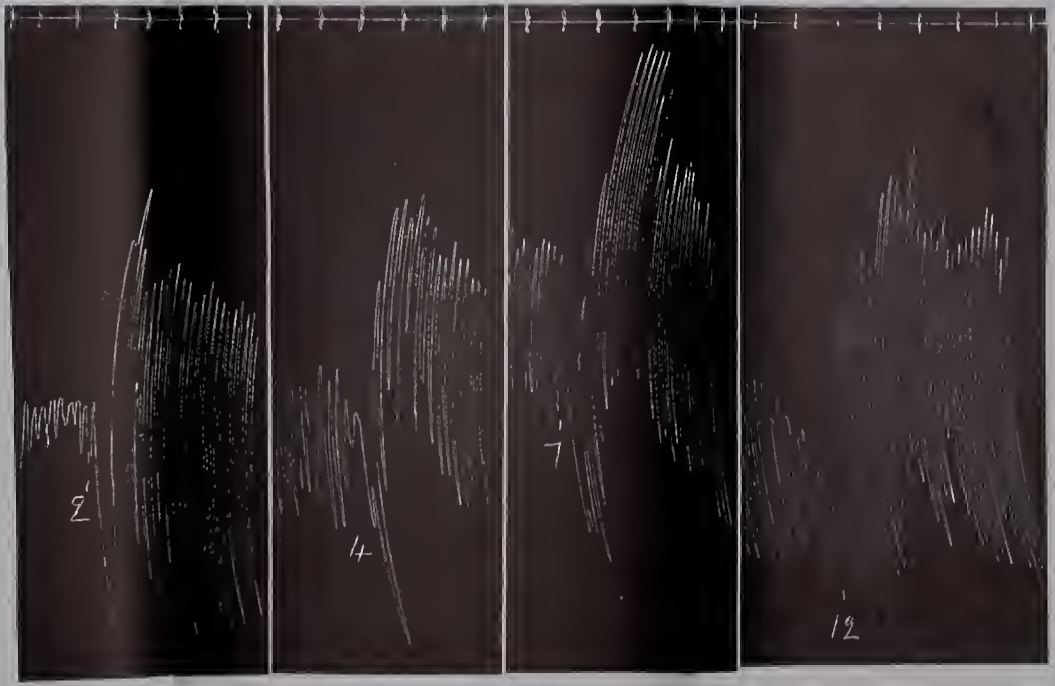

Fig. 19.

PLATE 19.

FIG. 20. Uterus. Rabbit hirudin material of Sept. 26. At 39 hirudin plasma, at 41 hirudin serum replaced Ringer. At 47 hirudin serum diluted with five volumes of Ringer, at 49 hirudin plasma diluted with five volumes of Ringer replaced Ringer. Weight of uterus preparation, 0.52 gm.

FIG. 21. Uterus. Dog hirudin material of Sept. 12. At 26 hirudin serum, and at 28 hirudin plasma replaced Ringer. At 30 Ringer solution was replaced by serum (from the dog's clot) diluted with its own volume of Ringer. The magnification of the lever was 2½ times less than that generally employed. Weight of uterus preparation, 0.74 gm.

FIG. 22. Uterus. Dog hirudin material of Aug. 29. At 24 hirudin plasma, at 26 the corresponding hirudin serum, and at 28 hirudin plasma replaced Ringer. The magnification was about half that usually employed. Weight of uterus preparation, 1 gm.

FIG. 23. The same material, the same uterus preparation, and the same magnification of the lever as those used for figure 22. At 30 the hirudin solution in Ringer diluted with two volumes of Ringer replaced Ringer. At 32 serum from dog defibrinated blood, at 36 adrenalin 1:1,000,000, and at 42 adrenalin 1:100,000 in Ringer replaced the Ringer solution.

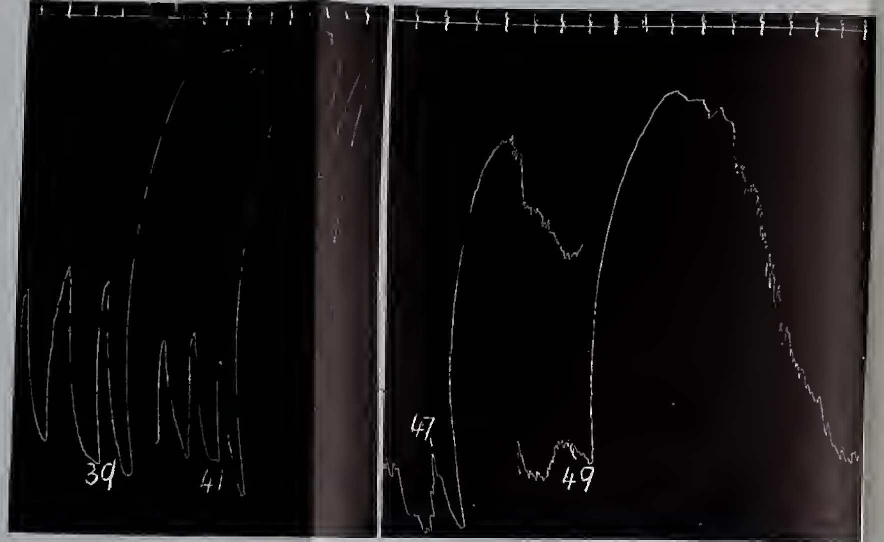

FIG. 20.

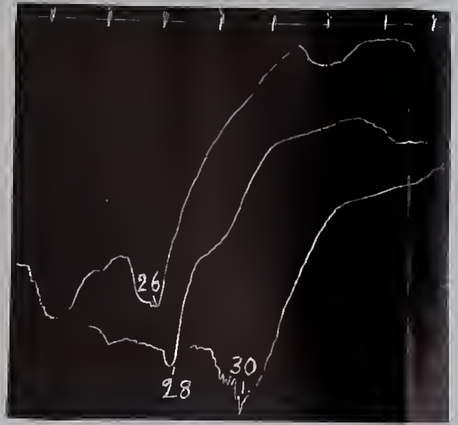

FIG. 21.

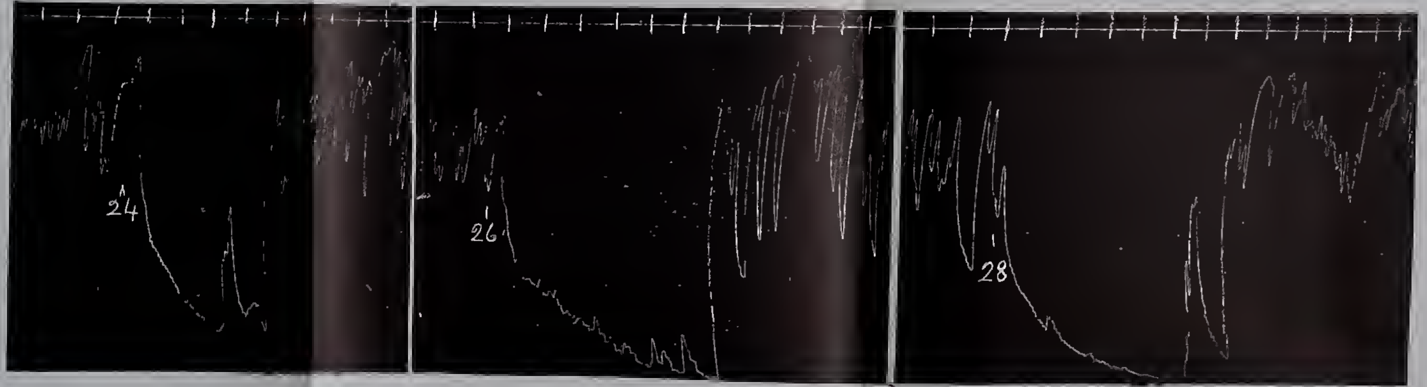

FIG. 22.

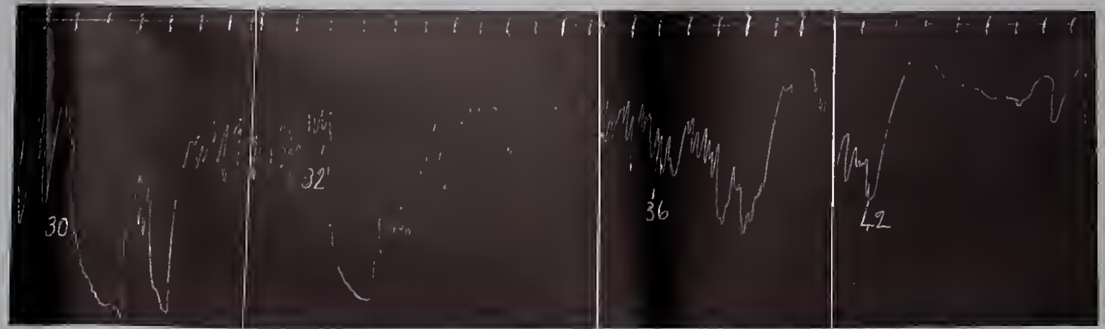

FIG. 23.

## PLATE 20.

FIG. 24. Intestine. Extracts and modifications of sheep serum of July 25. At 2 the serum, at 4. A(3) (a fraction prepared with alcohol), at 9 the serum diluted with four volumes of Ringer, and at 11 H(6) replaced Ringer. At 15 with a fresh intestine preparation Ringer was replaced by H(5). The point went above the drum. At 17 the serum diluted with four volumes of Ringer replaced Ringer when the segment was beating feebly and the tone was very small. At 18 the dilute serum was itself replaced by H(6) and this at 19 by H(5). The writing point went far above the drum and then fell abruptly, the contractions disappearing. At 20 H(5) was replaced by H(6) and the curve soon rose again.

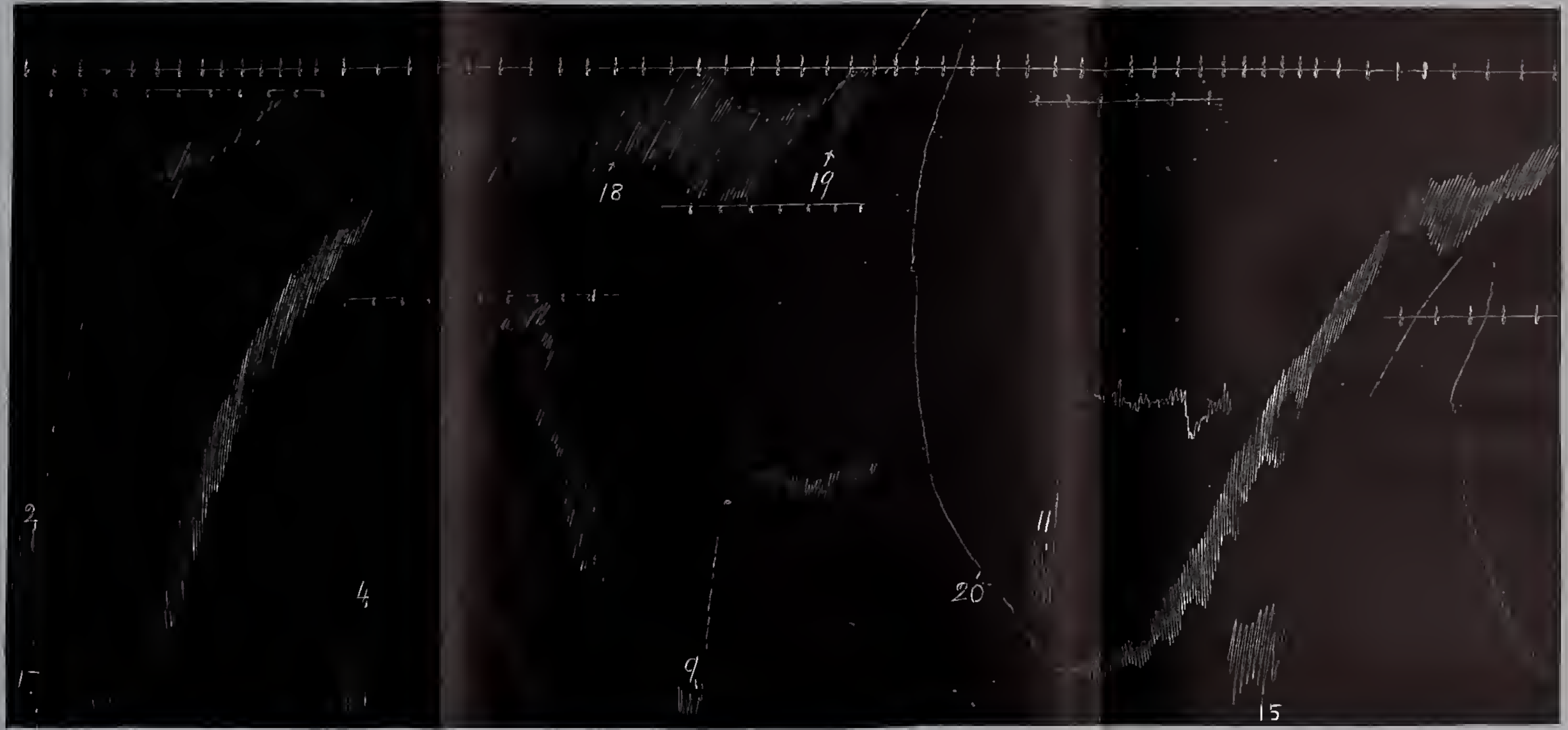

FIG. 24.

(Stewart and Zucker: Comparison of Action of Plasma and Serum.)

## PLATE 21.

FIG. 25. Uterus. Action of the same material used in figure 24. At 14 serum replaced Ringer. At 16 A(3) replaced Ringer. The writing point went above the drum. At 24 Ringer was replaced by H(6) and at 31 by H(5). At 32 a little more of H(5) was added to replace that carried away by the oxygen stream.

FIG. 26. Intestine. At 2 egg white diluted with nine volumes of Ringer, at 13 egg white diluted with two volumes of Ringer, and at 27 undiluted egg white (strained) replaced Ringer. At 29 serum from the rabbit's own defibrinated blood, at 31 egg white diluted with one volume of Ringer, and at 33 rabbit serum diluted with one volume of Ringer replaced the Ringer solution. Weight of intestine preparation, which was shorter than usual, 0.25 gm.

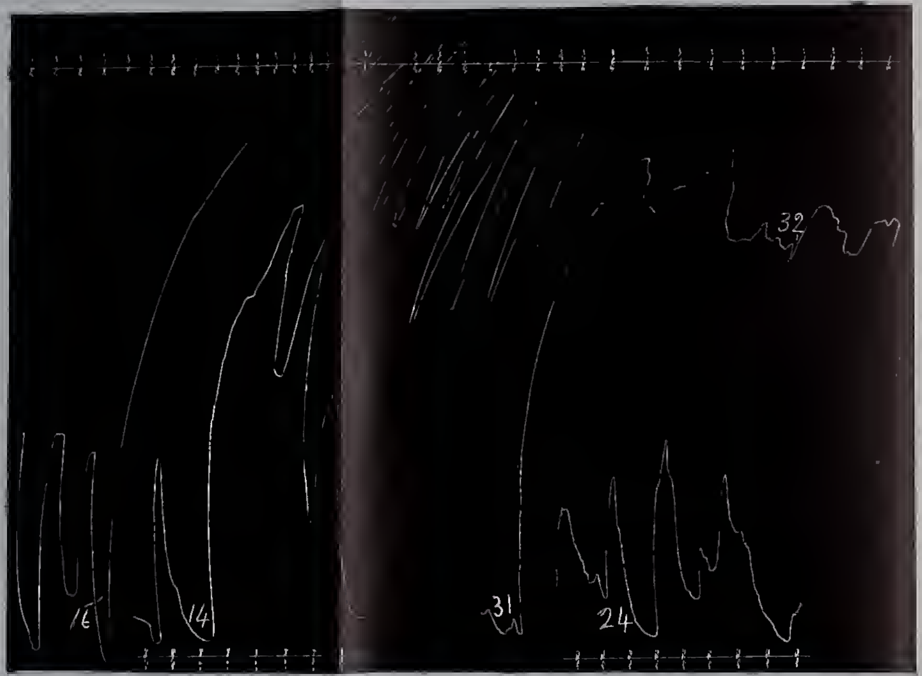

FIG. 25.

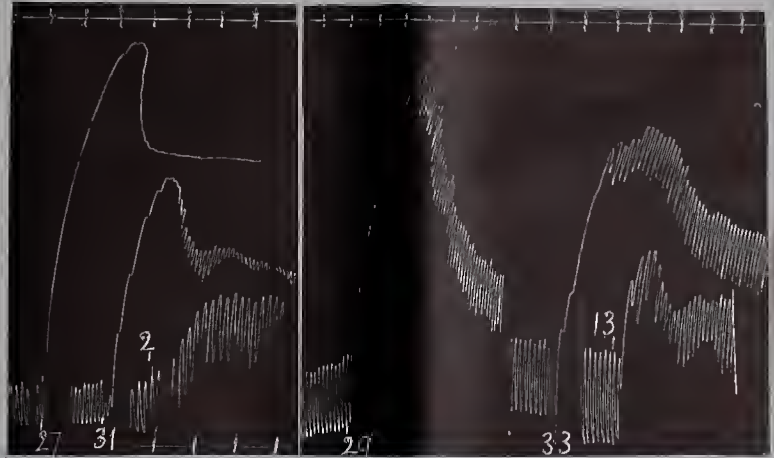

FIG. 26.

(Stewart and Zucker: Comparison of Action of Plasma and Serum.)

conceivable that the alterations in the cells which are connected with the development of this property may even be wholly or partially independent of the cell changes concerned in coagulation. In this case it might be possible to obtain blood which would clot without developing the pressor property.

The main results which seem to follow from our observations may be thus summarized:

1. The substance, or property, developed in shed blood by which it causes constriction of artery rings, is not developed, or at least not mainly developed, in connection with the actual change of fibrinogen to fibrin, since the constrictor action of different plasmas differs greatly, while the absence of coagulation is common to all.

2. The development of the constrictor substance, or property, is associated with changes undergone probably by formed elements of the blood when it is shed. These changes may be identical with the alterations, or with some of the alterations, preliminary to clotting. It is possible, however, that there may be changes connected with the development of the pressor property which, although concomitant with the liberation of the substances concerned in the production of thrombin, are yet quite independent of the clotting process. Our observations do not enable us to decide definitely between these possibilities.

3. The constrictor substance, or property, developed in shed blood acts especially on blood vessels and does not equally affect the other organs examined. This follows from the fact that a plasma and serum which differ markedly in their action on the blood vessels may have practically the same action on the intestine or uterus segments, an action which must therefore reside mainly, at least, in the original plasma itself.

4. The indication that the serum acts especially on blood vessels increases the interest of the suggestion that this action may play an important part in the prompt sealing of wounded vessels in addition to the mechanical effect of the clot, or by coming into operation before the clot has fully formed.

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# THE ACTION OF HYDROCELE FLUID AND CERTAIN OTHER PATHOLOGICAL LIQUIDS ON SOME OF THE OBJECTS USED IN BIOLOGICAL TESTS FOR EPINEPHRIN.*

By G. N. STEWART, M.D., AND T. F. ZUCKER, M.S.

*(From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University, Cleveland.)*

PLATES 22 TO 27.

The question whether the tone-increasing action of serum on intestine and uterus segments is due altogether, or mainly, to the same property of the serum which causes it to constrict artery rings and to slow the stream through the frog perfusion preparation of Laewen[1] was tested by comparing the effect of hydrocele fluid on the four preparations. The typical hydrocele fluid is a transudate which, although containing fibrinogen, does not clot spontaneously, or at least not readily, but can be made to clot by the addition of thrombin or such liquids as blood serum which contain thrombin. Its poverty in thrombin is related to its poverty in formed elements. If the development of the property in shed blood by which it causes constriction of blood vessels is connected with changes in the formed elements, this property should be absent or very slightly marked in hydrocele fluid. If in spite of a negative result with blood vessels hydrocele fluid were found to cause an action on intestine and uterus segments comparable in magnitude to that of serum, the conclusion would be decidedly strengthened that the action of serum on these latter objects is different in nature from its action on the blood vessel preparation. In the following experiment these expectations were realized.

Hydrocele fluid was obtained with such precautions as insured the complete absence of blood. The fluid was the typical non-inflammatory hydrocele fluid, perfectly clear and yellowish in color, and it

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* Received for publication, November 2, 1912.

[1] Stewart, G. N., and Zucker, T. F., *Jour. Exper. Med.*, 1913, xvii, 152.

remained free from the slightest clot for the whole period (over a week) during which it was kept in the ice chest. It was put on ice a very short time after being drawn. The total proteins were determined by precipitation with excess of alcohol.[2] The determination was made in duplicate on two samples of fifty cubic centimeters each. One yielded 2.37 grams, the other 2.38 grams; the protein content of 100 cubic centimeters was, therefore, 4.75 grams.

## RESULTS.

*Artery Rings.*—Hydrocele fluid caused no noticeable effect when it replaced Ringer solution (figure 1, observation 8), while serum from a patient in diabetic coma replacing the hydrocele fluid (observation 9) caused a prompt and large constriction.

*Frog Perfusion Preparation.*—The slowing produced by the hydrocele fluid was slight and transient in comparison with that caused by dog serum, and the original outflow was quickly and completely restored (figure 2). The initial elevation on the curve is simply due to the mechanical increase of pressure caused by the injection.

*Rabbit Intestine Preparation.*—The increase of tone caused by the hydrocele fluid when it replaced Ringer solution was not far from equal to that caused by serum from the rabbit's own blood (figure 3, observations 2 and 6). This is shown even more strikingly in figure 4, where at 8 rabbit serum replaced Ringer solution and was itself replaced at 9 by hydrocele fluid. Instead of a fall, as would have been the case had the tone-increasing power of the hydrocele fluid been decidedly inferior to that of the serum, a sharp rise is seen at 9 due probably merely to the renewal of the liquid, and the curve then soon falls to the same height as before 9. When the observations were made in the reverse order, an almost identical curve was obtained. At 11, figure 4, Ringer solution was replaced by hydrocele fluid and the fluid at 12 by rabbit serum. The same transient rise occurred at 12 followed by a fall to the level which was being maintained before 12, indicating that the hydrocele fluid and serum were about equally potent in maintaining the increased tone.

That the actual change of fibrinogen into fibrin has little, if any-

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[2] Hoppe-Seyler, F., Handbuch der physiologisch- und pathologisch-chemischen Analyse, 8th edition, Berlin, 1909, 662.

thing, to do with the tone-increasing property of shed blood for the intestine segments is illustrated by observations on a pleuritic exudate obtained from the right pleural cavity of J. S., a man aged twenty-two years. He had been ill for three weeks and complained of pain in the right side. The fluid was obtained free from blood and soon clotted into a jelly so that it could not be poured out of the bottle. The liquid from this clot was compared in a series of observations on the rabbit intestine segment with blood serum from the same rabbit. In figure 5 at 8 Ringer was replaced by the pleural liquid, and at 10 Ringer was replaced by rabbit serum. The increase of tone in 10 was distinctly greater than in 8. This result was obtained repeatedly on the same segment. Thus hydrocele fluid, in which no fibrin had been formed, had a tone-increasing power on the intestine equal to that exerted by rabbit serum; while the pleural liquid, after the formation of much fibrin, was inferior to rabbit serum in this regard. The liquid was richer in protein than the hydrocele fluid, containing 3.081 and 3.069 grams in two portions of fifty cubic centimeters, *i. e.,* 6.15 grams in 100 cubic centimeters. The pleuritic exudate probably was, or had been, richer in formed elements than the hydrocele fluid, so that if cell decomposition or changes of permeability in the cells are responsible for the tone-increasing property as regards the intestine that property ought to have been more strongly developed in the pleuritic fluid.

That the change of fibrinogen into fibrin is not necessarily associated with the development of the pressor property of shed blood for artery rings, any more than with its tone-increasing power for intestine and uterus, was demonstrated by observations on fluid from a cyst of the suprarenal gland. This fluid was unclotted when obtained, but could be made to clot in the thermostat. The action of the liquid before and after clotting was compared on sheep artery rings and rabbit intestine and uterus segments (figures 6 to 9), and was found to be practically identical. This was not because the epinephrin which it contained caused an effect so great as to obscure any difference which might have existed. For it was shown that the concentration of epinephrin present, although considerable (probably somewhat less than 1:1,000,000), produced a much smaller pressor effect on the rings and a much smaller increase of tone in the uterus segment than did the cyst liquid. The extremely prompt rise in the case of the uterus preparation, which is by no means peculiar to the action of this liquid, but is characteristic of the reaction of the uterus segments to serum, precludes the possibility that the tone-increasing property of the liquid is developed after contact with the tissue. It is only a matter of seconds when the rise begins. Further, after emptying the apparatus no clot could be seen.

Incidentally we have here an example of a plasma (an unclotted serous liquid) which presented no advantages over the corresponding serum (the cyst liquid after coagulation) for epinephrin-testing.

*Rabbit Uterus Preparation.*—On the uterus preparation of the same rabbit, a young non-pregnant adult whose intestine had been used for testing the hydrocele fluid, the tone-increasing power of the hydrocele fluid was distinctly less than that of the rabbit serum, so that when the hydrocele fluid was caused to replace the serum at 19, figure 10, the tone of the preparation was decidedly diminished. When hydrocele fluid replaced Ringer solution the increase of tone was inferior to that occasioned by the rabbit serum. Adrenalin 1:5,000,000 caused an increase of tone after a slight preliminary inhibition, adrenalin 1:50,000 a marked increase of tone without preliminary inhibition. After being kept four days in the ice chest, during which time it remained perfectly clear and there was no sign of bacterial growth, the hydrocele fluid was again tested on the uterus segment from an adult non-pregnant rabbit (figure 11). When the hydrocele fluid replaced Ringer, a rise of tone (observation 38) was obtained of about the same magnitude as that caused by adrenalin 1:1,000,000 (observation 41) or of hirudin whole blood from a dog (observation 30). The rise occasioned by the hydrocele fluid in this observation appears to be much more transient than is really the case, since the drum was moving more slowly than in the other observations. For the sake of comparison, observation 28, showing the effect of hirudin defibrinated blood replacing Ringer solution, is also reproduced. Observation 32 shows the action of the undiluted dog serum, without hirudin, and observation 34 that of the serum diluted with its own volume of Ringer. The hydrocele fluid produces a maximum increase of tone fully equal to that given by the diluted serum, although not quite so durable. It will be remarked that as regards its protein content it also resembles a somewhat diluted serum. This agrees very well with the view that the action of the hydrocele fluid on the intestine and uterus is mainly a general nutritive action of the same nature as that of the serum. If we conceive, on the other hand, the serum effect as being due to a special substance originally present in the blood plasma, it would be necessary to assume that this substance in passing into the hydrocele fluid was diluted in approximately the same degree as the plasma proteins.

With rings of sheep artery dog serum, without hirudin, and hirudin serum gave a very good rise. The dog hirudin plasma also caused a good rise, although not so great as that given by the sera.

The results of all the observations on the hydrocele fluid strengthened the conclusion that the constrictor action of serum for blood vessels is not a general action of the serum common to other serous liquids, but that it is due to a property developed in the shed blood which has a special relation to blood vessels.

Some observations were also made on ascitic fluid obtained twice, at an interval of seven weeks, from the same patient.

J. M., a man 50 years old, was admitted on July 6, 1912, to the City Hospital, suffering from cardiorenal disease (mainly renal) with a high systolic pressure (200 mm. of mercury), and general edema involving the face and the upper and lower limbs. The urine constantly contained albumen and casts. The left border of the cardiac dullness was two fingers' breadth outside the nipple line. On auscultation at the apex the first sound was impure, the second sound loud. In the aortic area the second sound was much accentuated and the pulmonary second was also accentuated. The abdomen was distended with free fluid.

On July 11, eight liters of liquid were removed from the abdomen, on August 19 more than seven liters, and on August 28, nearly two liters. The specimens were obtained quite free from blood. The action of the liquid obtained on July 11 and August 28 was determined on artery rings, and on segments of intestine and uterus. The protein in the fluid of July 11 was 0.637 gm. and 0.623 gm. in two portions of 50 c.c., *i. e.,* 1.26 gm. in 100 c.c. In the specimen of August 28 somewhat more protein was present, 1.055 and 1.029 gm. in two portions of 50 c.c., *i. e.,* 2.084 gm. in 100 c.c. Neither specimen showed any trace of clotting, although kept for several days.

With the artery rings no rise whatever was caused (figure 1, observation 10) whereas a serum used for comparison produced a very good rise (observation 11).

On the intestine preparation the effect was an increase of tone accompanied by an increase in the size of the rhythmical contractions. The effect was qualitatively similar to that produced by serum from the same patient, as shown in figure 12, for which the material of July 11 was used. At 4 Ringer solution was replaced by the ascitic fluid, and at 6 by serum from the patient's clotted blood obtained at the same time. The tone-increasing effect of the undiluted serum was decidedly greater than that of the peritoneal liquid.

Observations 8 to 14 (figure 12) were made with the object of determining in what dilution the serum effect would be approximately equal to that of the liquid. At 8 Ringer solution was replaced by serum diluted with two volumes of Ringer, at 12 by serum diluted with five volumes of Ringer, at 14 by ascitic fluid. The effect produced by the fluid was not very different, as regards increase of tone, from that produced by the serum diluted with five volumes of Ringer, and was superior, as regards increase in the size of the beats. Although no great stress should be laid on the coincidence, it may be noted that here also the pathological serous liquid exerted an action which, as compared with that of the serum, was approximately proportional to its content of protein.

On the uterus preparation (figure 13) from an adult non-pregnant rabbit the ascitic fluid (of July 11) produced a good increase of tone followed by complete relaxation (observation 21). The relaxation was especially evident when the fluid was added at a time when the uterus segment possessed a high initial tone (observation 38). At 39 it was particularly observed that relaxation was complete. The relaxation following the increase of tone was equally complete in observation 21 where the initial tone was low. The contractions ceased entirely during the relaxation.

On the uterus preparation from another rabbit (non-pregnant, small, and immature) the same phenomenon of relaxation with cessation of contraction was observed (figure 14, observation 24) succeeding a preliminary increase of tone. On the same segment the serum of the patient caused a good rise of tone which was sustained (observation 22).

The specimen of ascitic fluid obtained from the same patient on

August 28 caused a fair increase of tone in a rabbit intestine segment, although distinctly less than that produced by dog serum diluted with five volumes of Ringer solution.

On the uterus preparation from an adult non-pregnant rabbit the same inhibition succeeding the increase of tone which was observed with the other specimen of the fluid from this patient was noted. We are not in a position to state what the cause of this inhibition was. The other transudate examined (hydrocele fluid) also exhibited this effect, although to a smaller extent. It is a phenomenon which we have not observed with blood serum or plasma and is probably due to some constituent of the pathological liquids not present, or present in much smaller amount in blood. However this may be, the uterus observations again illustrate the point on which we have more than once insisted elsewhere, that we cannot postulate without experiment a similar action of a given serous liquid on different test objects any more than we can postulate a similar action of different serous liquids on one and the same test object. Each liquid must be investigated separately for each test object.

This point is well illustrated by the tracings reproduced in figures 15 and 16 in which the action of blood serum from a fatal case of diabetic coma is compared with that of dog serum (of September 12). In figure 15 with a rabbit intestine preparation, Ringer solution was replaced at 14 by the dog serum and at 16 by the diabetic serum. In figure 16 with the uterus preparation from the same rabbit (a non-pregnant adult), Ringer solution was replaced at 36 by the diabetic serum and at 38 by the same dog serum as was used in figure 15. The increase of tone in the uterus segment caused by the diabetic serum was much greater than that produced by the dog serum, whereas the dog serum occasioned a greater increase of tone in the intestine segment than the diabetic serum. On the artery rings the diabetic serum caused an unusually prompt and large effect.

The diabetic serum was obtained from a young woman twenty years old, a telephone operator. She collapsed from overwork and was treated by her physician for nervous breakdown for some weeks before she applied for treatment at Lakeside and Western Reserve University dispensary. On admission at the dispensary she showed tachycardia, exophthalmos, slight thyroid enlargement, and loss of weight. Sugar was present in the urine to the extent of 3 per cent.

The diagnosis made was Graves' Disease with glycosuria. She was put on a non-carbohydrate diet, and returned the following week slightly improved. There was still some sugar in the urine. The same treatment was continued. She returned the next week and said she was worse and entered the hospital, where she quickly developed well marked coma with a distinct odor of acetone in the breath. The non-carbohydrate diet was cancelled, but the coma progressively deepened and she died about twelve hours later without regaining consciousness. Ten hours before death 100 c.c. of blood were removed preparatory to the giving of sodium bicarbonate intravenously. This was the specimen of blood tested.

We are indebted to the staffs of Lakeside Hospital and the City Hospital, especially to Dr. Bucher of the City Hospital, for the material investigated.

## EXPLANATION OF PLATES.

All tracings are to be read from left to right. Time is marked in half minutes. Unless otherwise mentioned the liquid to be tested was always caused to displace the Ringer solution in which the preparation was immersed. Two c.c. of the liquid to be tested were always added, which was sufficient to displace the Ringer solution. The weight of the preparations, after drying with blotting paper, is given to facilitate comparison of the mass of tissue acted on, with the amount of active substance.

### PLATE 22.

FIG. 1. Sheep artery rings. At 8 hydrocele fluid replaced Ringer, at 9 serum from a patient in diabetic coma replaced the hydrocele fluid. The slight rise in the curve after 8 does not indicate a genuine constriction due to the hydrocele fluid. It was probably caused by accidental jarring. At 10 ascitic fluid from J. M. replaced Ringer, and was itself replaced at 11 by dog serum. The rise produced by the dog serum is more abrupt than was observed with any other serum except that from the case of diabetic coma.

FIG. 2. Frog perfusion preparation. Curves showing changes in outflow with hydrocele fluid and dog serum, each diluted with three volumes of Ringer solution. The number of drops per half minute is laid off along the vertical, and the time in half minutes along the horizontal axis. Each small division corresponds to a half minute. Twenty-one drops correspond to 1 c.c.

FIG. 3. Rabbit intestine. Weight of preparation, 0.27 gm. At 2 hydrocele fluid, at 4 serum from the rabbit's blood, and at 6 hydrocele fluid replaced Ringer.

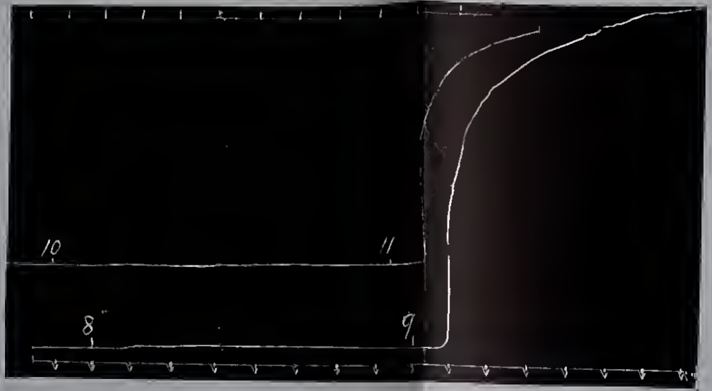

FIG. 1.

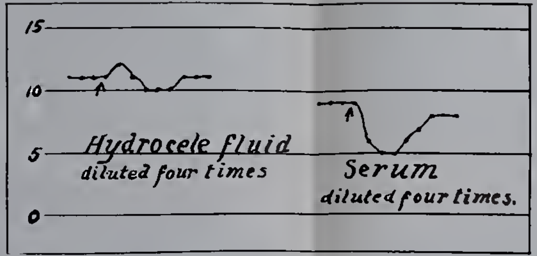

FIG. 2.

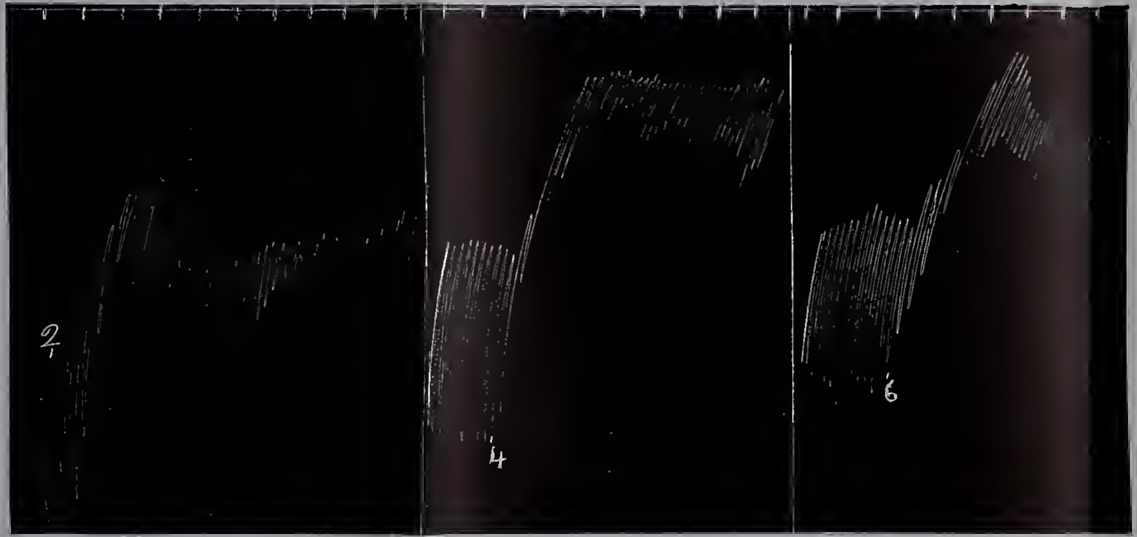

FIG. 3.

(Stewart and Zucker: Action of Hydrocele Fluid.)

### Plate 23.

Fig. 4. The same intestine preparation as that used for figure 3. At 8 Ringer was replaced by the rabbit's own serum, and this at 9 by hydrocele fluid. At 11 Ringer was replaced by hydrocele fluid, and this at 12 by rabbit serum.

Fig. 5. Intestine. Weight of preparation, 0.34 gm. At 8 serum from clotted pleural fluid (from J. S.) replaced Ringer. At 10 rabbit serum replaced Ringer.

Fig. 6. Sheep artery rings. Fluid from cyst of suprarenal gland. At 3 Ringer was replaced by the fluid which had clotted at 4 by the unclotted fluid at 5 by adrenalin solution in Ringer, 1 : 50,000 (freshly opened).

Fig. 7. Intestine preparation. Weight of preparation, 0.30 gm. At 8 Ringer was replaced by unclotted adrenal cyst liquid, at 10 by cyst liquid after it had clotted.

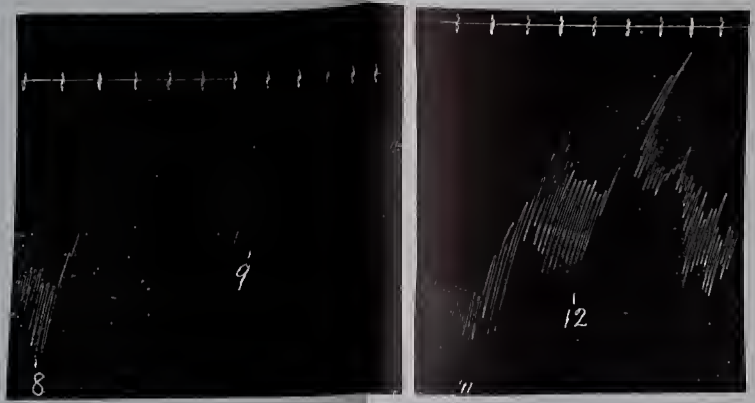

Fig. 4.

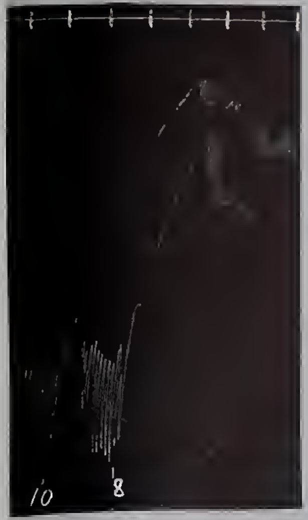

Fig. 5.

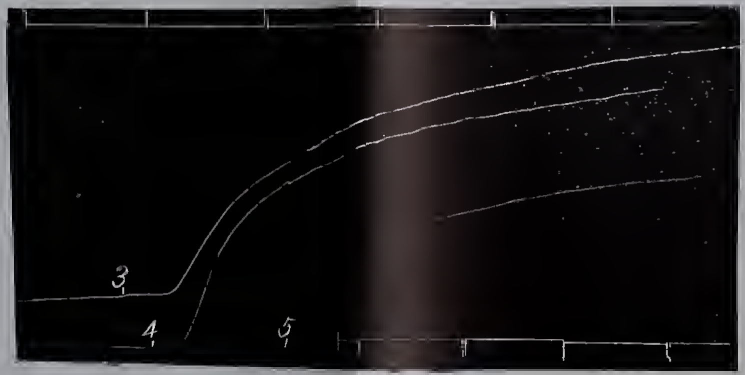

Fig. 6.

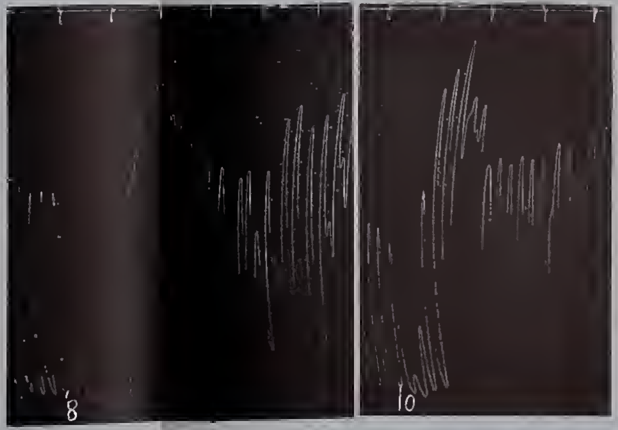

Fig. 7.

## Plate 24.

Fig. 8. Same intestine preparation as used in figure 7. At 12 Ringer was replaced by the unclotted cyst fluid, at 14 by the cyst fluid after clotting. At 16 sheep serum replaced Ringer and was itself replaced at 17 by the unclotted cyst fluid

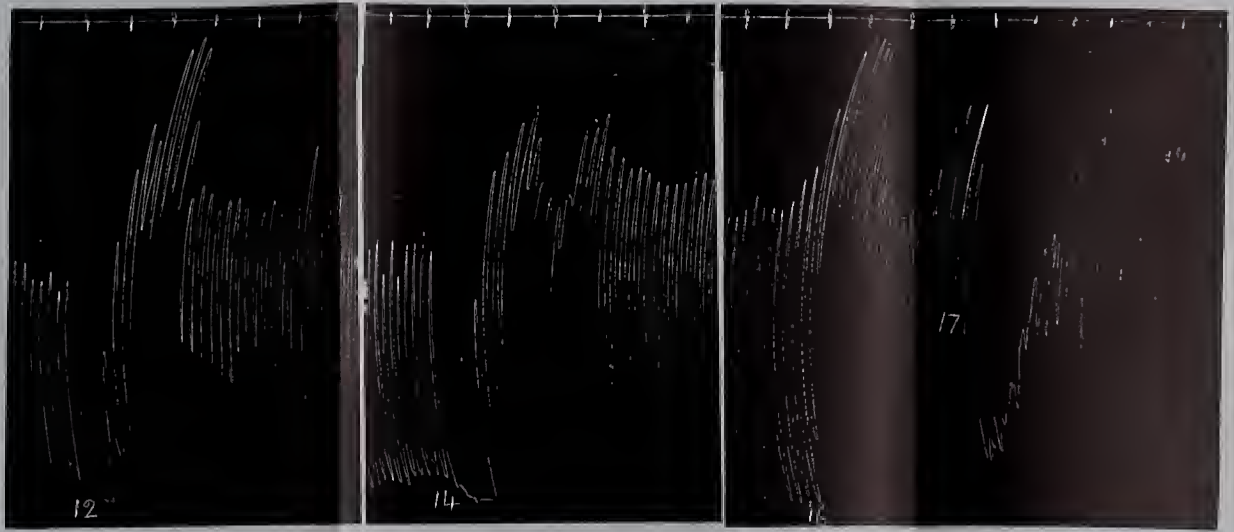

Fig. 8.

(Stewart and Zucker: Action of Hydrocele Fluid.)

## PLATE 25.

Fig. 9. Uterus preparation from an adult non-pregnant rabbit. Weight of preparation, 0.30 gm. At 28 Ringer was replaced by the unclotted cyst liquid, at 30 by the cyst liquid after it had clotted. The writing point went a little above the drum and then gradually came down. At 32 Ringer was again replaced by the unclotted cyst liquid.

Fig. 10. Uterus from a young non-pregnant rabbit. Weight of preparation, 0.17 gm. At 18 Ringer was replaced by rabbit serum and this at 19 by hydrocele fluid. At 20 additional hydrocele fluid was run in. The magnification was two thirds of that usually employed.

Fig. 11. Uterus from an adult non-pregnant rabbit. Weight of preparation, 0.71 gm. Magnification 2½ times less than that usually employed. Comparison of hydrocele fluid with material obtained from a dog on Sept. 10. At 28 Ringer was replaced by the dog's hirudin defibrinated blood, at 30 by the dog's whole hirudin blood, at 32 by undiluted serum from the dog's clot, at 34 by serum from the dog's clot diluted with its own volume of Ringer, at 38 by hydrocele fluid, and at 41 by adrenalin 1 : 1,000,000 in Ringer solution. The time trace above observation 28 shows that the drum was then moving much more slowly than during the other observations.

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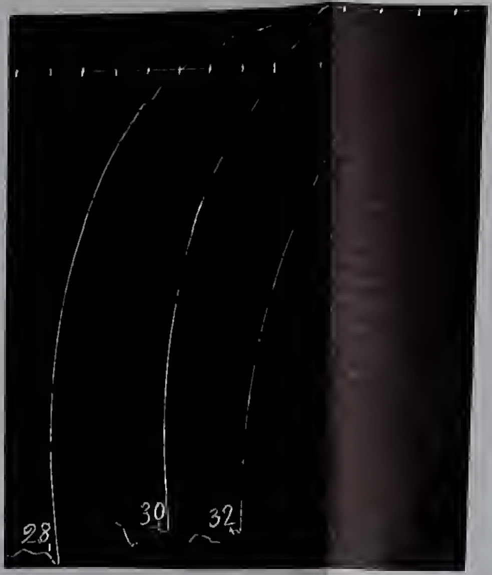

Fig. 9.

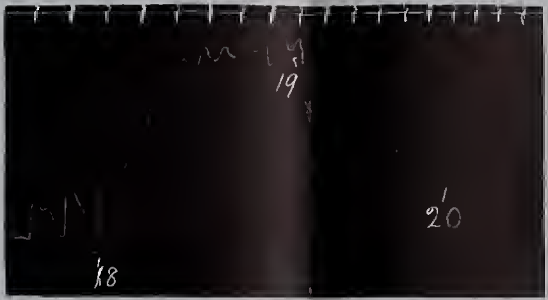

Fig. 10.

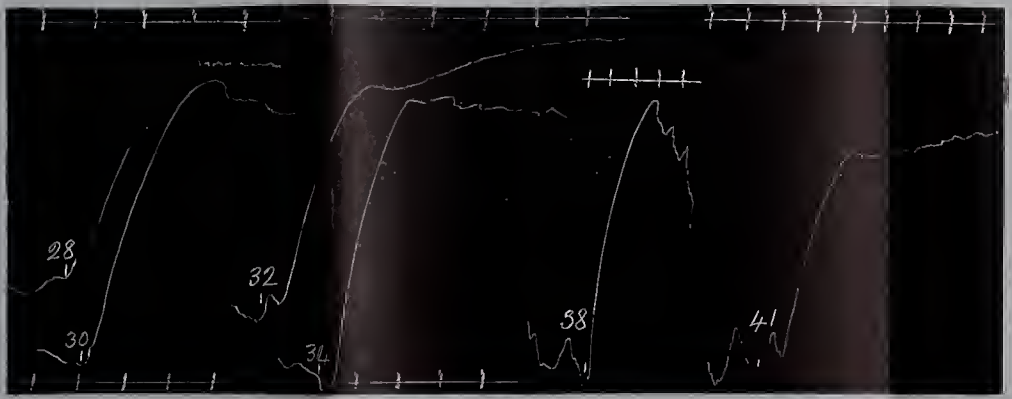

Fig. 11.

PLATE 26.

FIG. 12. Intestine. Weight of preparation, 0.25 gm. At 4 ascitic fluid from J. M., at 6 serum from J. M., at 8 his serum diluted with two volumes of Ringer, at 12 his serum diluted with five volumes of Ringer, and at 14 his ascitic fluid replaced Ringer.

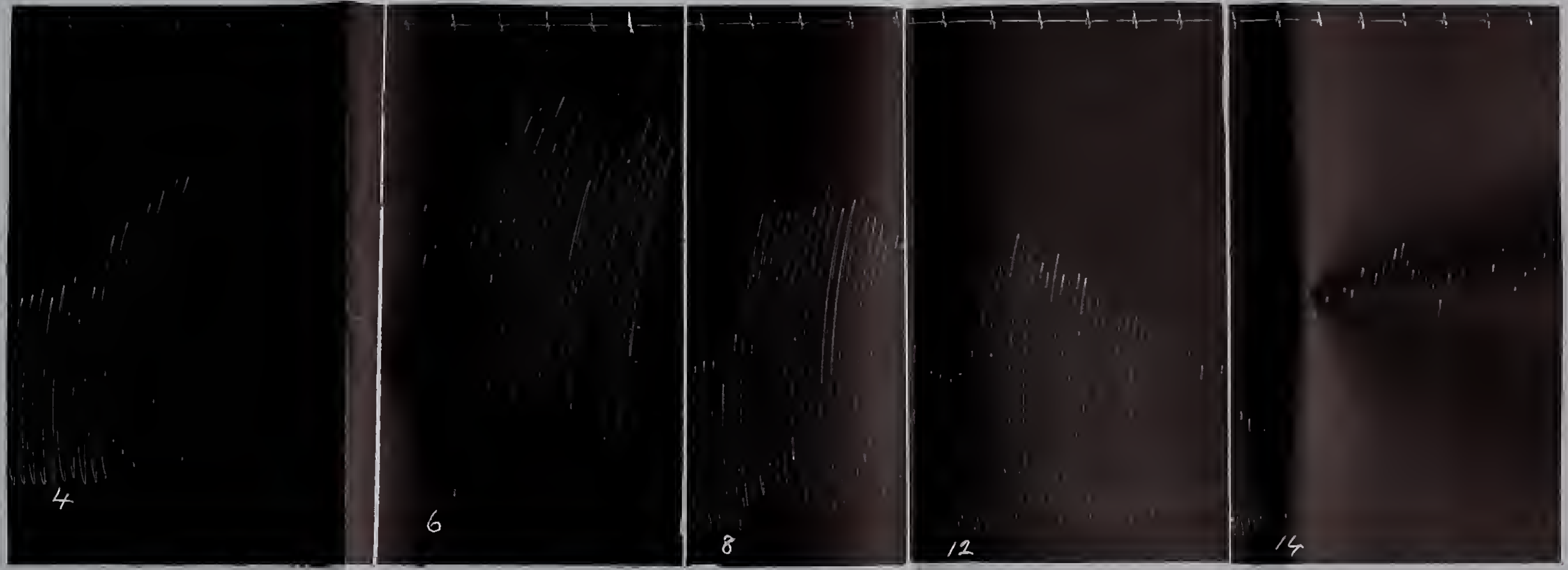

FIG. 12.

(Stewart and Zucker: Action of Hydrocele Fluid.)

## PLATE 27.

FIG. 13. Uterus from an adult non-pregnant rabbit. Weight of preparation, 0.55 gm. At 21 ascitic fluid from J. M. replaced Ringer. The initial tone of the uterus segment was small. At 38 the ascitic fluid replaced Ringer when the initial tone of the segment was high. At 39 the preparation was seen to be completely relaxed. The magnification was 2½ times less than that usually employed.

FIG. 14. Uterus preparation from a young non-pregnant rabbit. Weight of preparation, 0.45 gm. At 22 J. M.'s serum, diluted with five volumes of Ringer solution, and at 24 his ascitic liquid replaced Ringer.

FIG. 15. Intestine. Weight of preparation, 0.40 gm. At 14 serum from dog's clot, at 16 serum from a patient in diabetic coma replaced Ringer. The magnification was 2½ times less than that generally used.

FIG. 16. Uterus. Weight of preparation, 0.76 gm. At 36 serum from the patient in diabetic coma, and at 38 serum of the clot of the same dog that was used in figure 15 replaced Ringer. The magnification was the same as in figure 11.

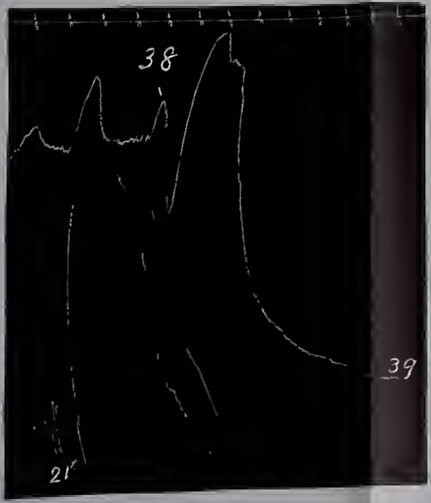

FIG. 13.

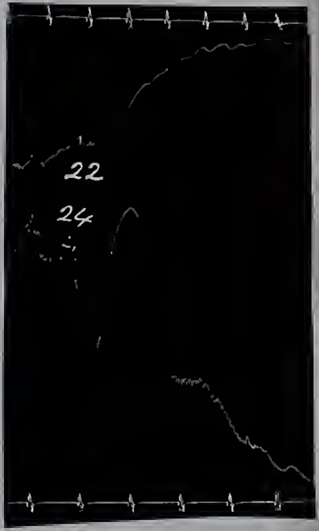

FIG. 14.

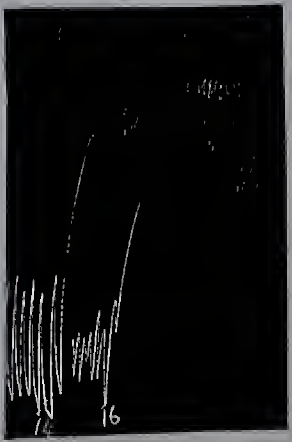

FIG. 15.

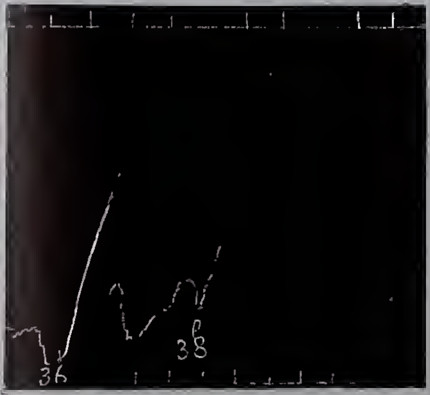

FIG. 16.

(Stewart and Zucker: Action of Hydropic Fluid.)

# STUDIES ON THE CIRCULATION IN MAN*

## V. EFFECT ON THE BLOOD FLOW IN THE HAND OF APPLYING DIFFERENT PRESSURES TO THE UPPER ARM; A CONTRIBUTION TO THE CLINICAL MEASUREMENT OF BLOOD-PRESSURE

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CLEVELAND, O.

### SECTION I. THE INFLUENCE ON THE FLOW IN THE HAND OF A PRESSURE IN THE ARMLET EQUAL TO THE SYSTOLIC ARTERIAL PRESSURE

The question has been often discussed whether in the clinical measurement of blood-pressure the pressure regarded as systolic pressure is actually sufficient to obliterate completely the lumen of the artery or only sufficient, without obliteration, to block the passage of the pulse-wave; but scarcely, so far as I am aware, has it been conclusively answered. I have put it to the test in the following way, using three of the recognized methods of estimating systolic pressure; that is to say, the determination of the pressure at which the radial pulse first reappears after extinction (*a*) by palpation, (*b*) by the stethoscope applied to the brachial artery at the elbow, (*c*) by the graphic record of the Erlanger apparatus. Broad armlets were employed.

I first attempted to compare the heat given off by one hand during compression of the upper arm, by a known pressure in the armlet steadily maintained, with that given off by the other hand, in which the blood was circulating freely. It is clear from the description of the method of estimating the blood-flow (Paper I) that for some time after complete cessation of the flow, the hand will continue to give off heat to the calorimeter since its interior is at a somewhat higher temperature than the calorimeter at the moment of introduction. If the circulation is interrupted for a sufficient time, however, the hand will eventually assume throughout its whole thickness the temperature of the calorimeter, and henceforth only the small amount of heat produced in the resting and asphyxiating hand will be given off. If we calculate the blood-flow in the hand after complete interruption of the circulation from the heat given off by the hand

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*Manuscript submitted for publication April 2, 1912.

*Paper I of this series, containing a description of the method, appeared in Heart, 1911, iii, 33; Paper II, Ibid. p. 76; Paper III, Am. Jour. of Physiol., 1911, xxviii, 133; Paper IV, Jour. of Pharmacol. and Exper. Therap., 1911, ii, 477.

TABLE 1 (EXPERIMENT 1).—SHOWING COURSE OF THE HEAT EXCHANGE WITH TOTAL INTERRUPTION OF CIRCULATION

| Time | Calorimeter Temperature | | | | | From Beginning of Experiment | | | | T—T′ | | H / T—T′ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Actual | | Corrected* | | | Increment of (corrected) temp. | | Heat Given Off | | | | | | |
| | R | L | R | L | Diff. | R | L | R | L | R | L | R | L | Diff. |
| 4.02 | 29.77 | 29.83 | ..... | ..... | .06 | ... | .... | ..... | ..... | .... | .... | ... | ..... | ... |
| 4.03 | 29.89 | 29.93 | 29.90 | 29.94 | .04 | .13 | .11 | 454 | 384 | 6.92 | 6.87 | 666 | 567 | 10† |
| 4.04 | 29.99 | 30.02 | 30.01 | 30.06 | .03 | .24 | .21 | 838 | 733 | 6.87 | 6.83 | 122 | 107 | 15 |
| 4.05 | 30.09 | 30.11 | 30.12 | 30.14 | .03 | .35 | .31 | 1,221 | 1,072 | 6.82 | 6.78 | 179 | 158 | 21 |
| 4.06 | 30.16 | 30.19 | 30.19 | 30.23 | .04 | .42 | .40 | 1,466 | 1,396 | 6.79 | 6.74 | 216 | 206 | 10 |
| 4.07 | 30.25 | 30.25 | 30.29 | 30.31 | .02 | .52 | .48 | 1,815 | 1,675 | 6.74 | 6.71 | 269 | 250 | 19 |
| 4.11 | 30.39 | 30.50 | 30.47 | 30.60 | .13 | .70 | .77 | 2,443 | 2,687 | 6.67 | 6.59 | 366 | 408 | 42 |
| 4.13 | 30.43 | 30.64 | 30.52 | 30.76 | .24 | .75 | .93 | 2,617 | 3,246 | 6.65 | 6.52 | 393 | 498 | 105 |
| 4.15½ | 30.47 | 30.81 | 30.58 | 30.96 | .38 | .81 | 1.13 | 2,827 | 3,944 | 6.63 | 6.44 | 426 | 612 | 186 |
| 4.17 | 30.48 | 30.90 | 30.61 | 31.07 | .46 | .84 | 1.23 | 2,932 | 4,293 | 6.63 | 6.39 | 442 | 672 | 230 |
| 4.19 | 30.48 | 31.02 | 30.62 | 31.21 | .58 | .85 | 1.38 | 2,966 | 4,816 | 6.63 | 6.33 | 447 | 761 | 314 |
| 4.21 | 30.49 | 31.13 | 30.65 | 31.34 | .69 | .88 | 1.51 | 3,141 | 5,270 | 6.62 | 6.28 | 464 | 839 | 375 |
| 4.22¼ | 30.495 | 31.20 | 30.67 | 31.42 | .75 | .90 | 1.59 | 3,141 | 5,550 | 6.62 | 6.24 | 474 | 889 | 415 |
| 4.25 | 30.49 | 31.37 | 30.69 | 31.62 | .93 | .92 | 1.79 | 3,211 | 6,247 | 6.62 | 6.16 | 485 | 1,014 | 529 |
| 4.26¼ | 30.495 | 31.41 | 30.70 | 31.68 | .98 | .93 | 1.85 | 3,246 | 6,456 | 6.62 | 6.14 | 490 | 1,051 | 561 |
| 4.28 | 30.52 | 31.51 | 30.74 | 31.80 | 1.06 | .97 | 1.97 | 3,385 | 6,875 | 6.61 | 6.09 | 512 | 1,129 | 617 |
| 4.29 | 30.53 | 31.55 | 30.76 | 31.85 | 1.09 | .99 | 2.02 | 3,455 | 7,050 | 6.60 | 6.07 | 523 | 1,161 | 638 |
| 4.30 | 30.565 | 31.58 | 30.78 | 31.89 | 1.11 | 1.01 | 2.06 | 3,525 | 7,189 | 6.59 | 6.05 | 535 | 1,188 | 653 |
| 4.31 | 30.57 | 31.64 | 30.82 | 31.96 | 1.14 | 1.05 | 2.13 | 3,664 | 7,434 | 6.58 | 6.02 | 557 | 1,235 | 678 |
| 4.32 | 30.59 | 31.67 | 30.85 | 32.00 | 1.15 | 1.08 | 2.17 | 3,769 | 7,573 | 6.57 | 6.01 | 574 | 1,260 | 686 |
| 4.33 | 30.595 | 31.73 | 30.86 | 32.07 | 1.21 | 1.09 | 2.24 | 3,804 | 7,818 | 6.57 | 5.98 | 579 | 1,307 | 728 |
| 4.34 | 30.63 | 31.755 | 30.90 | 30.11 | 1.21 | 1.13 | 2.28 | 3,954 | 7,957 | 6.55 | 5.96 | 604 | 1,335 | 731 |
| 4.35 | 30.67 | 31.80 | 30.95 | 32.16 | 1.21 | 1.18 | 2.33 | 4,118 | 8,132 | 6.53 | 5.94 | 631 | 1,369 | 738 |
| 4.36 | 30.695 | 31.83 | 30.98 | 32.20 | 1.22 | 1.21 | 2.37 | 4,223 | 8,271 | 6.52 | 5.92 | 648 | 1,397 | 749 |
| 4.37½ | 30.75 | 31.90 | 31.05 | 32.29 | 1.24 | 1.28 | 2.46 | 4,467 | 8,585 | 6.49 | 5.89 | 688 | 1,458 | 770 |
| 4.39 | 30.82 | 31.95 | 31.13 | 32.36 | 1.23 | 1.36 | 3.53 | 4,746 | 8,830 | 6.46 | 5.86 | 735 | 1,507 | 772 |
| 4.40 | 30.87 | 31.99 | 31.19 | 32.41 | 1.22 | 1.42 | 2.58 | 4,956 | 9,004 | 6.43 | 5.84 | 771 | 1,542 | 771 |
| 4.41 | 30.905 | 32.035 | 31.24 | 32.46 | 1.22 | 1.47 | 2.63 | 5,130 | 9,178 | 6.41 | 5.82 | 800 | 1,577 | 777 |
| 4.42 | 30.94 | 32.05 | 31.28 | 32.49 | 1.21 | 1.51 | 2.66 | 5,270 | 9,283 | 6.40 | 5.81 | 823 | 1,598 | 775 |
| 4.50 | 30.87 | 31.97 | ..... | ..... | .... | | | | | | | | | |

*For loss of heat to room and in accordance with the assumption that the left hand was as large as the right.

†The first five numbers in this column are excesses of R over L, the rest excesses of L over R.

("blood-flow" in this case being merely a phrase which it might be convenient to use for the sake of uniformity), it will come out greater immediately after the interruption of the circulation than later on, and will diminish steadily. Where the flow is partially interrupted the slope of temperature in the hand may be expected to be altered also but to a less extent than after total interruption. The flow calculated in the usual way will then come out somewhat greater than it actually is, but by and by a steady slope of temperature will be again established and henceforth the calculated and the actual flows will be identical. When the circulation is reestablished through the hand in which it has been completely stopped, some of the heat brought to the hand by the blood will at first go to heat the interior of the hand till the steady state has again been reached, and the calculated flow at first will therefore be less than the real flow. Usually in the following pages the heat exchange has alone been given

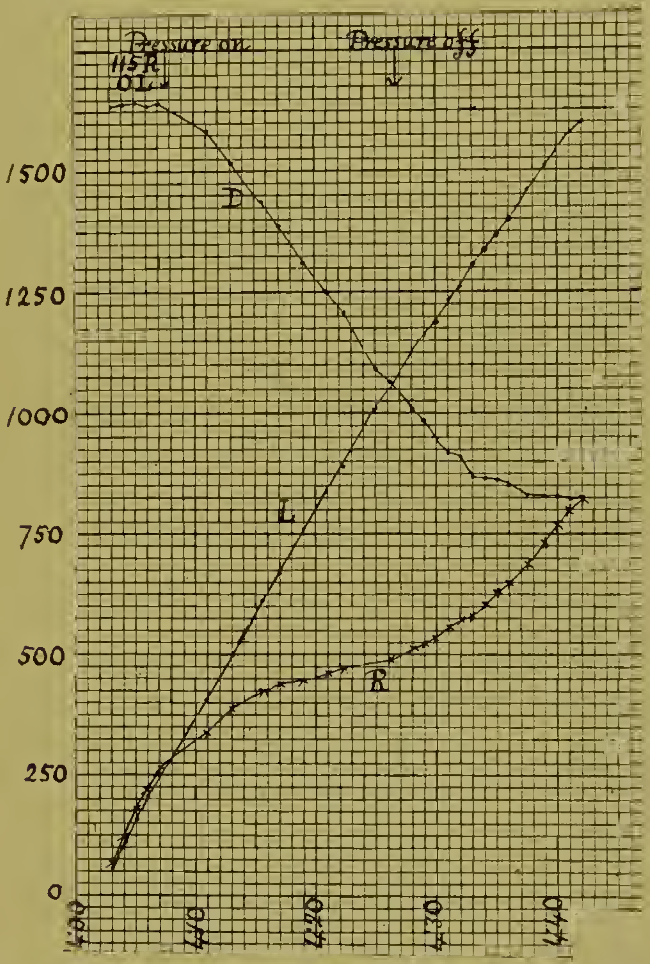

Fig. 1.—R and L represent the change with the time measured from the beginning of the experiment in the quantity $\frac{H}{T-T'}$, which measures the blood-flow, and D, the difference in this quantity for the two hands (Exper. 1).

when the circulation was totally or partially interrupted, and has not been translated into "blood-flow." Experiment 1 will illustrate the course of the heat exchange with total interruption of the circulation. The results of this experiment, as displayed in Table 1, are plotted in two different ways in Figures 1 and 2.

*Experiment 1.*—M. C., a healthy man, 23 years old. 3:45 p. m., systolic arterial pressure in right arm taken with Riva-Rocci apparatus (armlet 12.5 cm. broad), 115 mm., in two observations (subject sitting). 3:49 p. m.: Hands put into bath, armlet being left on right arm but disconnected from manometer. Mouth temperature 36.75 C.; pulse 88 (sitting); room 25.1 C. 4:00 p. m.: Calorimeter R. 29.675 C., L 29.74 C.; 3,015 c.c. water in each calorimeter. 4:01 p. m.: Hands put into calorimeters, right, as always, into R, left into L. Armlet on right arm at once connected with manometer but no pressure made as yet. At 4:07½ made pressure of 115 mm. mercury on right arm. At 4:26¾ released pressure. At 4:37 p. m. removed armlet. At 4:42 removed hands from calorimeter. At end of experiment the right hand felt a little swollen. Its volume was 493 c.c.; that of left 459 c.c.

In Figure 1 the quantity $\frac{H}{T - T'}$ (where T is the temperature of the arterial blood, T′ the mean temperature of the calorimeter, and H the heat given off to the calorimeter), reckoning from the first observation after the hands were introduced into the calorimeters, is plotted for each hand along the vertical and the time along the horizontal axis. Thus, R and L in Figure 1 represent the way in which the quantity $\frac{H}{T - T'}$, increases with the time for right and left hand respectively. When the flow is unhindered this quantity, multiplied by the reciprocal of the specific heat of blood, corresponds to the amount of blood which has passed through the hand from the beginning of the observations to the time represented by any point on the curve. $\frac{H}{T - T'}$, is the quantity which measures the blood-flow on the assumption that the slope of temperature throughout the hand remains steady. As already pointed out, this assumption is only justified for a hand through which the blood is steadily flowing and not for a hand in which the flow has suffered an abrupt change or been interrupted altogether. Since in accordance with the plan of the experiments, interference with the circulation was always caused in at least one hand, it was considered that for most purposes it would be more instructive to compare the amounts of heat given off by the hands without reference to the factor T — T′. For this comparison it is enough to take the calorimeter temperatures at each time, corrected for loss of heat to the room and for the difference in the volume of the hands, and in all of the following Figures, including Figure 2, this has alone been done. With unobstructed circulation, equal increments of temperature (thus corrected) correspond to equal blood-flows in the two hands so long as the mean temperatures of the two calorimeters remain the same. When the temperature of one calorimeter rises above that of the other the blood-flow through the hand whose calorimeter shows excess of temperature increases more rapidly than the excess of temperature. For most of the experiments, however, the temperature of the calorimeters remained so nearly the same that when the curves representing the increase of temperature of the calorimeters (with unobstructed flow) are parallel to each other no serious error is introduced by assuming that the blood-flow in the two hands is then the same. Still, to see whether with such changes of temperature as occurred in the calorimeter in any one experiment there would be any marked difference between the curves when plotted in the two ways, both methods were adopted for Experiment 1, where the difference of temperature was greater than in any of the other experiments and where accordingly the difference between the curves yielded by the two methods would be at a maximum. Inspection of Figures 1 and 2 shows that the results are not obscured whichever graphic method is adopted.

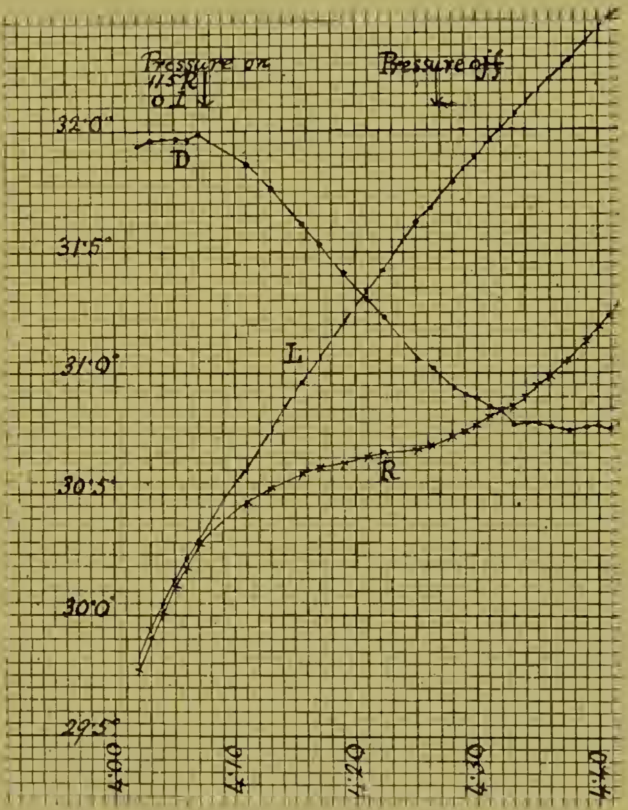

Fig. 2.—R. and L (corrected) calorimeter temperatures for right and left hand, respectively (in Experiment 1), D, differences in these temperatures. R had not become quite horizontal when the pressure was releasd, i. e., the right hand was still giving off some heat to its calorimeter (not, however, due to a flow of blood in it, as shown in other experiments).

Table 2 of the same experiment shows the quantities of heat given off by the two hands per 100 c.c. of hand per minute for seven periods, making in the aggregate a little over thirty-four minutes. The first period (five minutes) was before pressure had been raised in the armlet, and the last three periods after the pressure had been released.

For a period of four and one-half minutes beginning two to three minutes after establishment in the cuff on the right arm of a pressure equal to the systolic pressure, the heat given off by the right hand was only 18.9 small calories per 100 c.c. per minute as compared with 73.6 calories before the pressure was established. For the next five and one-half minutes the heat given off by the right hand was 8.9; for the next five and one-quarter minutes, pressure being still maintained on the right arm, only 6.7 small calories per 100 c.c. per minute. If we take the heat production of the resting hand at 1 small calorie per 100 c.c. per minute,[1] this would leave, on the assumption that a steady slope of temperature had been again at this time established in the right hand, 5.7 small calories per 100 c.c. per minute as the heat given off by the blood passing through the hand, corresponding to a flow of almost exactly 1 gm. per 100 c.c. per minute or one-twelth of the original amount. It is quite certain, however, that at the moment when the pressure in the cuff was released the steady state had not yet been attained in the hand and therefore the conclusion may safely be drawn, even from this experiment, which was not carried on for a sufficiently long time to afford a decisive test, that the flow, if any, in the right hand must have been less than one-twelfth of the unhindered flow.

TABLE 2 (EXPERIMENT 1).—HEAT GIVEN OFF PER 100 C.C. OF HAND PER MINUTE

| Period in Mins | Heat Given Off in Small Cal.* | | Mean Temp. of Calorim's | | Blood-Flow in Grams* | | Pressure in Armlet | |
|---|---|---|---|---|---|---|---|---|
| | R | L | R | L | R | L | R | L |
| 5 | 73.6 | 70.9 | 30.01 | 30.04 | 12.14 | 11.74 | 0 | 0 |
| 4½ | 18.9 | 59.5 | 30.43 | 30.66 | ..... | 10.84 | 115 | 0 |
| 5½ | 9.0 | 74.7 | 30.48 | 30.97 | ..... | 13.11 | 115 | 0 |
| 5¼ | 6.7 | 47.4 | 30.49 | 31.27 | ..... | 9.91 | 115 | 0 |
| 4 | 18.6 | 37.7 | 30.56 | 31.59 | ..... | 8.11 | 0 | 0 |
| 4 | 24.8 | 37.7 | 30.64 | 31.75 | ..... | 8.38 | 0 | 0 |
| 6 | 35.4 | 35.2 | 30.82 | 31.94 | ..... | 8.13 | 0† | 0 |

*Per 100 c.c. of hand per minute. †Armlet off.

As already pointed out, on reestablishing the circulation a portion of the heat brought by the blood must go to warm the hand itself until a steady slope of temperature has been once more established. The heat given off by the hand whose circulation was stopped must therefore for a time be less than that given off by the other. This is clearly shown in Experiment 1, Table 2. For the first four minutes after release of the pressure the right hand gave off only 18.6 calories per 100 c.c. per minute, about half the amount lost by the left hand. Even ten minutes later it is probable that the steady state had not been attained since the flow, reckoned on the assumption that it had been reached, was only 6.63 gm. for the right hand against 8.13 gm. for the left. It must be remembered

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[1] Heart, 1911, iii, 44.

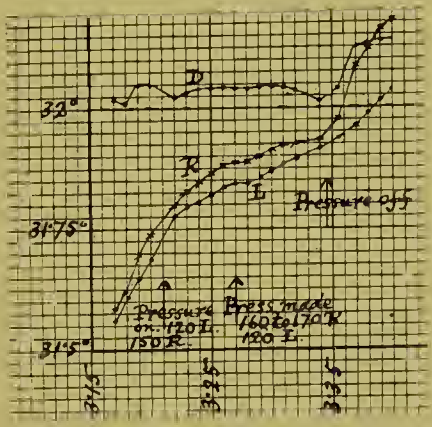

Fig. 3.—R and L (corrected) calorimeter temperatures for right and left hands in Experiment 2, and D, differences in these temperatures.

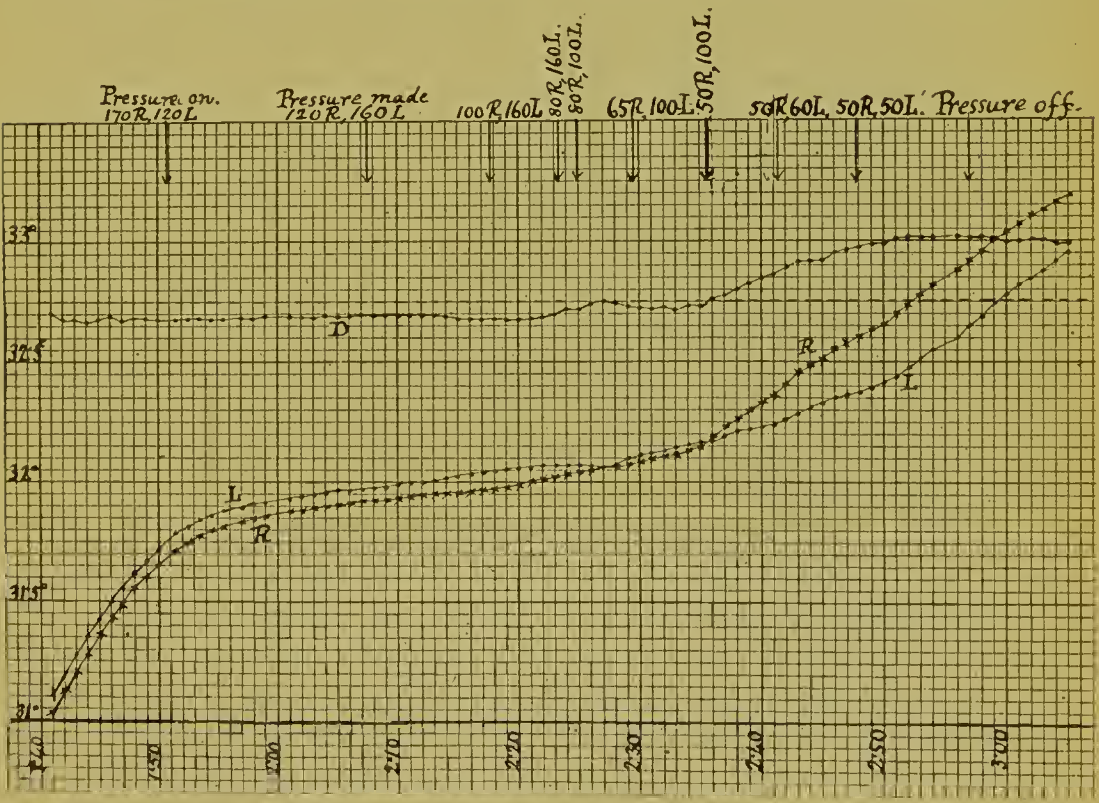

Fig. 4.—R, L and D have the same signification as in Fig. 3 but are plotted from Experiment 3, made with another person. So long as the pressures are equal to or greater than systolic pressure, D is horizontal and R and L parallel.

that the flow is directly proportional to the heat given off and inversely proportional to the difference between the mean temperature of the calorimeter and the temperature of the arterial blood coming to the hand.

It was soon seen that it would be too tedious for the subject to continue the experiment in this form for a long enough time to insure that the loss of heat from the occluded hand had become steady. Accordingly another method was adopted which did not require that the observation should be carried to this point. This method had the further advantage that no assumption as to the amount of heat produced in the hand in the absence of a circulation needed to be made. The systolic pressure was first determined in the two arms. Then after a period during which the unobstructed blood-flow was measured in the two hands, a pressure equal to the systolic pressure was established in one armlet and simultaneously a pressure considerably higher than the systolic pressure, and therefore a pressure certainly enough to obliterate completely the arteries was made in the other. Comparison of the heat given off by the two hands would now show whether there was any excess in the case of the first hand. If not, it is clear that no blood could be passing through that hand. Experiments 2 and 3 are examples on two different healthy persons. The results of these experiments are graphically represented in Figures 3 and 4, respectively. In these figures the temperatures of the two calorimeters (corrected for loss of heat and for the difference in the size of the two hands), and the differences at each minute between the two temperatures, as shown in Tables 3 and 4, are plotted along the vertical, and the time along the horizontal axis. It will be observed that with systolic pressure on one arm and a very decidedly greater pressure on the other the curves of heat loss for the two hands remain practically parallel. This could not be the case unless the circulation was completely stopped at the lower of the two pressures. It is only when the pressure on one arm is diminished distinctly below the systolic pressure (Fig. 4) that the curves begin to diverge.

In Table 5 of Experiment 3, wherein the average heat exchange of the hands is calculated for four-minute to ten-minute periods throughout the experiment, the same result is brought out in another way. For example, the difference between the heat loss of the right and left hands remains practically unchanged by the application of pressures of 120 and 170 respectively to the right and left arms. This could not be if the flow was completely stopped in one hand and not in the other. The difference increases very markedly when the pressure is made 50 on the right and 100 on the left arm.

*Experiment 2.*—Armlets were placed on both arms of M. C. Systolic pressure in right arm 120 mm. and 118 mm. in two observations with Riva-Rocci apparatus. The subject was sitting in a high chair with his hands hanging down exactly in the position they would occupy in the calorimeters. It was always in this position

TABLE 3 (EXPERIMENT 2).—EFFECT OF DIFFERENT PRESSURES ON HEAT GIVEN OFF BY HANDS AS SHOWN BY CALORIMETER TEMPERATURES

| Time | Calorimeter Temperature | | | | |
|---|---|---|---|---|---|
| | Actual | | Corrected | | |
| | R | L | R | L | Diff |
| 3.17 | 31.58 | 31.56 | ..... | ..... | 0.02 |
| 3.18 | 31.61 | 31.60 | 31.62 | 31.61 | 0.01 |
| 3.19 | 31.68 | 31.63 | 31.70 | 31.65 | 0.05 |
| 3.20 | 31.71 | 31.66 | 31.74 | 31.69 | 0.05 |
| 3.22 | 31.75 | 31.73 | 31.80 | 31.78 | 0.02 |
| 3.23 | 31.77 | 31.74 | 31.83 | 31.79 | 0.04 |
| 3.24 | 31.78 | 31.76 | 31.85 | 31.80 | 0.05 |
| 3.25 | 31.79 | 31.75 | 31.87 | 31.82 | 0.05 |
| 3.26 | 31.795 | 31.755 | 31.88 | 31.84 | 0.04 |
| 3.27 | 31.79 | 31.755 | 31.89 | 31.85 | 0.04 |
| 3.28 | 31.78 | 31.745 | 31.88 | 31.84 | 0.04 |
| 3.29 | 31.785 | 31.745 | 31.90 | 31.85 | 0.05 |
| 3.30 | 31.79 | 31.75 | 31.91 | 31.87 | 0.04 |
| 3.31 | 31.79 | 31.755 | 31.92 | 31.88 | 0.04 |
| 3.32 | 31.785 | 31.76 | 31.93 | 31.89 | 0.04 |
| 3.34 | 31.77 | 31.765 | 31.93 | 31.92 | 0.01 |
| 3.35½ | 31.80 | 31.77 | 31.98 | 31.94 | 0.04 |
| 3.37 | 31.895 | 31.78 | 32.09 | 31.96 | 0.13 |
| 3.38 | 31.92 | 31.80 | 32.12 | 31.99 | 0.13 |
| 3.40 | 31.95 | 31.82 | 32.16 | 32.02 | 0.14 |
| 3.39 | 31.96 | 31.83 | 32.18 | 32.04 | 0.14 |
| 3.52½ | 31.84 | 31.72 | ..... | ..... | .... |

BLE 4 (EXPERIMENT 3).—CHANGES IN HEAT GIVEN OFF BY HANDS UNDER VARYING PRESSURES AS SHOWN BY CALORIMETER TEMPERATURES

| me | Calorimeter Temperature | | | | | Time | Calorimeter Temperature | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Actual | | Corrected | | | | Actual | | Corrected | | |
| | R | L | R | L | Diff | | R | L | R | L | Diff |
| 0 | 31.02 | 31.09 | ..... | ..... | .... | 2.24 | 31.51 | 31.57 | 32.03 | 32.06 | 0.03 |
| 1 | 31.03 | 31.11 | 31.04 | 31.12 | 0.08 | 2.25 | 31.505 | 31.56 | 32.04 | 32.07 | 0.03 |
| 2 | 31.09 | 31.16 | 31.11 | 31.18 | 0.07 | 2.26 | 31.505 | 31.54 | 32.05 | 32.06 | 0.01 |
| 3 | 31.17 | 31.24 | 31.21 | 31.28 | 0.07 | 2.27 | 31.50 | 31.54 | 32.06 | 32.06 | 0.00 |
| 4 | 31.23 | 31.31 | 31.28 | 31.36 | 0.08 | 2.28 | 31.50 | 31.55 | 32.07 | 32.08 | 0.01 |
| 5 | 31.30 | 31.37 | 31.36 | 31.43 | 0.07 | 2.29 | 31.50 | 31.555 | 32.08 | 32.10 | 0.02 |
| 6 | 31.37 | 31.43 | 31.44 | 31.50 | 0.06 | 2.30 | 31.50 | 31.56 | 32.09 | 32.11 | 0.02 |
| 7 | 31.395 | 31.47 | 31.48 | 31.55 | 0.07 | 2.31 | 31.495 | 31.56 | 32.10 | 32.12 | 0.02 |
| 8 | 31.465 | 31.53 | 31.56 | 31.62 | 0.06 | 2.32 | 31.50 | 31.56 | 32.12 | 32.14 | 0.02 |
| 9 | 31.495 | 31.56 | 31.60 | 31.66 | 0.06 | 2.33 | 31.495 | 31.565 | 32.13 | 32.16 | 0.03 |
| 50 | 31.535 | 31.61 | 31.65 | 31.72 | 0.07 | 2.34 | 31.50 | 31.555 | 32.14 | 32.15 | 0.01 |
| 51½ | 31.58 | 31.66 | 31.72 | 31.79 | 0.07 | 2.35 | 31.50 | 31.555 | 32.15 | 32.16 | 0.01 |
| 52½ | 31.60 | 31.67 | 31.75 | 31.81 | 0.06 | 2.36 | 31.52 | 31.555 | 31.19 | 32.18 | 0.01* |
| 53½ | 31.62 | 31.69 | 31.78 | 31.84 | 0.06 | 2.37 | 31.55 | 31.56 | 32.23 | 32.20 | 0.03 |
| 54½ | 31.625 | 31.70 | 31.80 | 31.87 | 0.07 | 2.38 | 31.575 | 31.565 | 32.26 | 32.21 | 0.05 |
| 55½ | 31.63 | 31.705 | 31.81 | 31.88 | 0.07 | 2.39 | 31.595 | 31.56 | 32.30 | 32.22 | 0.08 |
| 57 | 31.63 | 31.70 | 31.83 | 31.89 | 0.06 | 2.40 | 31.62 | 31.57 | 32.33 | 32.24 | 0.09 |
| 58 | 31.635 | 31.71 | 31.85 | 31.91 | 0.06 | 2.41 | 31.63 | 31.565 | 32.36 | 32.25 | 0.11 |
| 59 | 31.635 | 31.705 | 31.86 | 31.92 | 0.06 | 2.42 | 31.67 | 31.57 | 32.41 | 32.26 | 0.15 |
| 01 | 31.63 | 31.70 | 31.88 | 31.94 | 0.06 | 2.43 | 31.71 | 31.59 | 32.46 | 32.29 | 0.17 |
| 02 | 31.62 | 31.69 | 31.88 | 31.94 | 0.06 | 2.44 | 31.72 | 31.60 | 32.48 | 32.31 | 0.17 |
| 03 | 31.615 | 31.69 | 31.89 | 31.95 | 0.06 | 2.45 | 31.735 | 31.605 | 32.51 | 32.33 | 0.18 |
| 04 | 31.615 | 31.685 | 31.90 | 31.96 | 0.06 | 2.46 | 31.77 | 31.615 | 32.55 | 32.35 | 0.20 |
| 05 | 31.61 | 31.685 | 31.91 | 31.97 | 0.06 | 2.47 | 31.78 | 31.61 | 32.58 | 32.36 | 0.22 |
| 06 | 31.605 | 31.675 | 31.91 | 31.97 | 0.06 | 2.48 | 31.80 | 31.62 | 32.61 | 32.38 | 0.23 |
| 07 | 31.605 | 31.67 | 31.93 | 31.98 | 0.05 | 2.49 | 31.815 | 31.625 | 32.64 | 32.40 | 0.24 |
| 08 | 31.595 | 31.66 | 31.93 | 31.98 | 0.05 | 2.50 | 31.82 | 31.63 | 32.65 | 32.41 | 0.24 |
| 09 | 31.58 | 31.65 | 31.93 | 31.98 | 0.05 | 2.51 | 31.855 | 31.65 | 32.70 | 32.44 | 0.26 |
| 10 | 31.58 | 31.65 | 31.94 | 31.99 | 0.05 | 2.52 | 31.88 | 31.67 | 32.74 | 32.47 | 0.27 |
| 11 | 31.575 | 31.645 | 31.94 | 31.99 | 0.05 | 2.53 | 31.91 | 31.70 | 32.78 | 32.51 | 0.27 |
| 12 | 31.57 | 31.64 | 31.95 | 32.00 | 0.05 | 2.54 | 31.935 | 31.73 | 32.82 | 32.56 | 0.26 |
| 13 | 31.565 | 31.635 | 31.96 | 32.01 | 0.05 | 2.56 | 31.98 | 31.76 | 32.87 | 32.60 | 0.27 |
| 14 | 31.55 | 31.63 | 31.96 | 32.02 | 0.06 | 2.57 | 32.01 | 31.805 | 32.91 | 32.65 | 0.26 |
| 15 | 31.54 | 31.63 | 31.96 | 32.03 | 0.07 | 3.58 | 32.04 | 31.83 | 32.96 | 32.69 | 0.27 |
| 16 | 31.535 | 31.625 | 31.96 | 32.03 | 0.07 | 2.59 | 32.08 | 31.875 | 33.01 | 32.75 | 0.26 |
| 17 | 31.53 | 31.62 | 31.97 | 32.04 | 0.07 | 3.00 | 32.10 | 31.90 | 33.03 | 32.78 | 0.25 |
| 18 | 31.525 | 31.615 | 31.98 | 32.05 | 0.07 | 3.01 | 32.13 | 31.93 | 33.07 | 32.82 | 0.25 |
| 19 | 31.515 | 31.61 | 31.98 | 32.05 | 0.07 | 3.02 | 32.15 | 31.94 | 33.10 | 32.84 | 0.26 |
| 20 | 31.515 | 31.605 | 31.99 | 32.06 | 0.07 | 3.03 | 32.16 | 31.95 | 33.13 | 32.87 | 0.26 |
| 21 | 31.515 | 31.60 | 32.00 | 32.06 | 0.06 | 3.04 | 32.18 | 31.99 | 33.16 | 32.92 | 0.24 |
| 22 | 31.51 | 31.595 | 32.01 | 32.07 | 0.06 | 3.05 | 32.20 | 32.01 | 33.19 | 32.95 | 0.24 |
| 23 | 31.51 | 31.585 | 32.02 | 32.07 | 0.05 | 3.29 | 31.915 | 31.74 | ..... | ..... | .... |

*From this to the end of the table the differences represent excesses of temperature of R. over L.: before his excesses of temperature of L. over R.

that the pressures were determined in this series of experiments. Disconnected armlets from manometers and put hands in bath at 2:45 p. m.; mouth temperature 36.75; rectal 37.225; pulse 87; room temperature 24.0. At 2:56¼ p. m. hands put into calorimeters; as always, 3,015 c.c. water in each. Between 3:20¼ and 3:21 p. m. a pressure of 120 mm. mercury was established in left armlet and 150 mm. in right. At 3:27 p. m. increased pressure in right to 160 to 170. At 3:34½ p. m. pressure released in both cuffs. At 3:40 p. m. hands taken out of calorimeters. Volume of right hand 465 c.c.; of left 465 c.c.

*Experiment 3.*—C. B., normal man, aged 23 years. Systolic pressure (with Riva-Rocci apparatus): right arm 114 and 113 in two observations; left arm 116, 118 and 116 in three observations. Subject sitting with hands in position they occupy when in calorimeters. Hands put in bath at 1:30 p. m., after disconnecting manometers. Mouth temperature 36.75 C., rectal 37.2 C., pulse 104. Room temperature 23.8 C. to 24.3 C. throughout experiment. At 1:40⅓ p. m. hands put into calorimeters, 3,015 c.c. water in each. Between 1:50½ and 150 5/6 p. m. a pressure of 120 mm. mercury was established in the left armlet and 170 mm. in right. These pressures were maintained (within 2 mm.) till 2:07½ p. m., when the pressure in the left armlet was made 160 mm. and that in the right 120 mm. No change till 2:17¾ p. m., when the pressure in the right was lowered to 100 mm.; that in left remaining 160 mm. It was soon noticed that the right forearm became red. The contrast in color between it and the left became striking. At 2:23⅓ p. m. lowered pressure in right to 80 mm., and at 2:24 5/6 p. m. lowered pressure in left to 100 mm. At 2:29½ p. m. made pressure in right 65 mm. keeping left at 100 mm. At 2:35½ p. m. pressure in right reduced to 50 mm. At 2:41½ p. m. pressure in left reduced to 60 mm., keeping right at 50 mm. At 2:47 5/6 p. m. pressure was also made 50 mm. in left. At 2:57 pressure released completely in both armlets. At 3:05 p. m. hands removed from calorimeters. Systolic pressure again measured. Right brachial 116 and 117 in two observations; left 112, 114, 116 in three observations. Volume of right hand 445 c.c., of left 442 c.c. As both hands felt swollen, the volume was again measured at 5:00 p. m. and found to be 410 c.c. for right, and 405 c.c. for left.

TABLE 5 (EXPERIMENT 3).—QUANTITY OF HEAT GIVEN OFF BY HANDS UNDER VARYING PRESSURES

| Period in Minutes | Heat Given Off (Small) Cal. per 100 c.c. Hand per Min. | | | Mean Temp. of Calorim's. | | Pressure on Arm | |
|---|---|---|---|---|---|---|---|
| | R | L | Diff. | R | L | R | L |
| 9 | 57.04 | 56.51 | 0.53 | 31.28 | 31.32 | 0 | 0 |
| 4 | 20.42 | 19.63 | 0.79 | 31.61 | 31.68 | 170 | 120 |
| 5½ | 9.89 | 8.48 | 1.41 | 31.63 | 31.71 | 170 | 120 |
| 6 | 6.28 | 5.37 | 0.91 | 31.62 | 31.69 | 170 | 120 |
| 9 | 3.91 | 5.62 | 1.71* | 31.57 | 31.64 | 120 | 160 |
| 5 | 7.53 | 4.42 | 3.11 | 31.52 | 31.60 | 100 | 160 |
| 10 | 9.53 | 9.08 | 0.45 | 31.50 | 31.56 | 80 | 100 |
| 5 | 28.54 | 11.16 | 17.38 | 31.58 | 31.56 | 50 | 100 |
| 5 | 28.54 | 16.30 | 12.24 | 31.73 | 31.59 | 50 | 60 |
| 9 | 29.53 | 26.94 | 2.59 | 31.91 | 31.71 | 50 | 50 |
| 8 | 29.85 | 31.26 | 1.41* | 32.11 | 31.91 | 0 | 0 |

*Excess of left over right. The other numbers in this column are excesses of right over left.

TABLE 6 (EXPERIMENT 4).—CALORIMETER TEMPERATURES WITH PRESSURES BELOW SYSTOLIC PRESSURE

| Time | Calorimeter Temperature | | | | |
|---|---|---|---|---|---|
| | Actual | | Corrected | | |
| | R | L | R | L | Diff |
| 3.35 | 29.67 | 29.655 | ..... | ..... | 0.015* |
| 3.36 | 29.73 | 29.73 | 29.742 | 29.744 | 0.002 |
| 3.37 | 29.81 | 29.82 | 29.834 | 29.848 | 0.014 |
| 3.38 | 29.89 | 29.88 | 29.926 | 29.922 | 0.004* |
| 3.42½ | 30.02 | 30.04 | 30.11 | 30.145 | 0.035 |
| 3.44 | 30.03 | 30.11 | 30.138 | 30.236 | 0.098 |
| 3.45½ | 30.035 | 30.17 | 30.161 | 30.317 | 0.156 |
| 3.47 | 30.05 | 30.28 | 30.194 | 30.448 | 0.254 |
| 3.48½ | 30.05 | 30.36 | 30.212 | 30.549 | 0.337 |
| | ..... | ..... | ..... | ..... | .... |
| 3.50 | 30.05 | 30.43 | 30.23 | 30.64 | 0.41 |
| 3.51½ | 30.06 | 30.48 | 30.258 | 30.711 | 0.453 |
| 3.53 | 30.08 | 30.53 | 30.296 | 30.782 | 0.486 |
| 3.54 | 30.095 | 30.55 | 30.323 | 30.816 | 0.493 |
| 3.55 | 30.11 | 30.62 | 30.35 | 30.90 | 0.55 |
| 3.57 | 30.16 | 30.69 | 30.424 | 30.998 | 0.574 |
| 3.58 | 30.185 | 30.725 | 30.461 | 31.047 | 0.586 |
| 3.59 | 30.21 | 30.74 | 30.498 | 31.08 | 0.582 |
| 4.00 | 30.25 | 30.80 | 30.55 | 31.15 | 0.60 |
| 4.12 | 30.11 | 30.64 | ..... | ..... | ..... |

*Excess of R. over L. The other numbers in this column are excesses of L. over R.

## SECTION II. THE INFLUENCE ON THE FLOW IN THE HANDS OF PRESSURES IN THE ARMLETS LESS THAN THE SYSTOLIC PRESSURE

If the blood-flow is completely arrested at a pressure equal to the systolic pressure, it is a question of some interest how it increases as the pressure in the cuff is lowered from the systolic pressure, or, what probably comes to the same thing, how the flow diminishes as the pressure in the cuff is raised from zero toward the systolic pressure.

In Experiment 4 the heat given off by the right hand was compared with that given off by the left when a pressure somewhat below the systolic pressure was maintained on the right arm while the flow through the left arm and hand was unobstructed. The results are graphically shown in Figure 5, constructed from Table 6.

TABLE 7 (EXPERIMENT 4).—HEAT GIVEN OFF UNDER PRESSURES BELOW SYSTOLIC PRESSURE

| Period in Mins. | Heat Given Off in Small Cals.* | | Mean Temp. of Calorimeters | | Blood-Flow in gm.* | | Pressure in Armlet | |
|---|---|---|---|---|---|---|---|---|
| | R | L | R | L | R | L | R | L |
| 3 | 63.4 | 69.9 | 29.78 | 29.77 | 10.46 | 11.53 | 0 | 0 |
| 4½ | 13.3 | 52.8 | 30.04 | 30.16 | .... | 9.25 | 90 | 0 |
| 3 | 8.7 | 50.1 | 30.05 | 30,36 | ..... | 9.07 | 90 | 0 |
| 3½ | 19.1 | 41.9 | 30.09 | 30.55 | ..... | 7.8 | 0 | 0 |
| 5 | 29.8 | 39.6 | 30.18 | 30.71 | ..... | 6.8 | 0 | 0 |

*Per 100 c.c. of hand per minute.

*Experiment 4.*—M. C., Armlet of Erlanger sphygmomanometer adjusted on right arm but not yet connected with manometer. At 3:22 p. m. hands put into bath; at 3:33½ p. m. hands put into calorimeters and armlet on right arm connected with manometer, but no pressure yet produced. Between 3:39 and 3:39½ p. m. made pressure of 90 mm. mercury on right arm and maintained it until 3:50½ p. m., when pressure was released. Hands removed from calorimeters at 4:00 p. m. Systolic pressure in right arm 100 to 105 mm. in seven separate observations. Volume of right hand 465 c.c.; of left 435 c.c.; pulse 76 (sitting); mouth temperature 36.5 C.; 3,015 c.c. water in each calorimeter.

If we compare Experiments 1 and 4, done on the same man on successive days, it is seen that with a pressure of from 10 to 15 mm. below the systolic pressure the heat given off was reduced nearly as much as with a pressure equal to the systolic pressure. In other words, a pressure in the cuff 10 or 15 mm. below the systolic pressure constituted an almost impervious block. Thus in Table 7, Experiment 4, for the first period of four and one-half minutes after the establishment of the pressure the heat given off by the right hand fell from 63.4 small calories per 100 c.c. of hand per minute, before the pressure was raised, to 13.3, and then, in the second period of three minutes, to 8.7, or less than one-seventh of the initial amount. As already remarked, the decrease in the blood-flow must be still greater. This experiment was done by the first method, and as previously explained, it could not be satisfactorily continued for a time long enough to eliminate entirely the effect due to change in the slope of temperature in the hand after the circulation was interfered with. In all the other experiments cited in this section of the paper the effect of a pressure below the systolic pressure on the heat given off by one hand was compared with the effect of a pressure equal to, or greater than, the systolic pressure on the other hand. Thus, in Experiment 5 with a mean systolic pressure of 113 mm. in the right brachial and 118 in the left, the heat given off by the two hands was compared when the pressure in the left armlet was made 120 (enough to stop the flow completely) and in the right 65 mm. In the first ten minutes after the establishment of the pressure the heat given off by the left hand was reduced to between one-sixth and one-seventh of the initial amount, while that given off by the right was reduced only to a little less than one-half. The blood-flow in the right hand (on the assumption that the initial slope of temperature in the right hand remains undisturbed, i. e., without allowing for any cooling of the hand, which would, of course, in any case be much less than for the hand in which the circulation was completely interrupted) works out at 7.45 gm. per 100 c.c. of hand per minute for the ten-minute period as compared with 14.07 gm. before pressure was made. So that the handicap

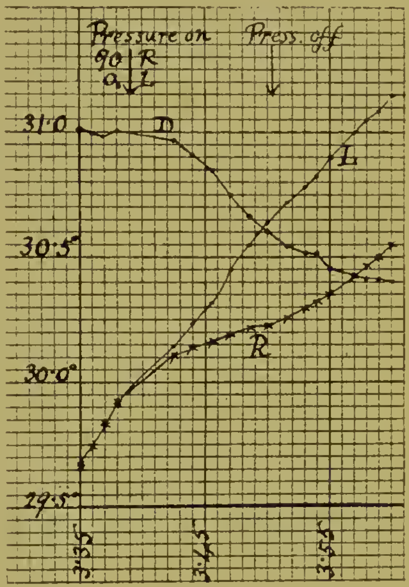

Fig. 5.—R and L (corrected) calorimeter temperature of right and left hand, respectively; D differences between those temperatures. The curves are plotted from Experiment 4.

of 65 mm. pressure in the armlet did not cut down the circulation quite to half. Probably the reduction was even considerably less than this. For on releasing the pressure, the flow, at least during the eight minutes for which it was still observed, did not regain its original volume, averaging only 9.38 gm. per 100 c.c. per minute for the right hand. Although a portion of this decline in flow is only apparent, and due to reheating of the hand, the fact that the flow in the left hand almost recovered its original ratio to that in the right within the eight-minute period indicates that the flow was in any case diminishing throughout the latter part of the experiment, and a portion of the diminution produced in the heat exchange of the right hand during the pressure period may be attributed to this cause.

*Experiment 5.*—M. C. Hands put in bath at 2:08 p. m. Put armlets on (Riva-Rocci on right, Erlanger on left) and measured systolic pressure with hands in bath. Right arm 112, 114 mm. in two observations; left arm 116, 120; mouth temperature 36.8 C. Hands put into calorimeters at 2:27½ p. m., 3,015 c.c. water in each. At 2:37½ p. m. got up pressure of 65 mm. mercury in right and 120 mm. in left, and maintained these pressures till 2:50½ p. m., when they were released. At 2:59 p. m. removed hand from calorimeters, dried and again measured systolic pressure in right arm (112 mm.). Volume of right hand 480 c.c.; of left 461 c.c. Room 24.2 C.

TABLE 8.—HEAT GIVEN OFF DURING EXPERIMENT 5

| Period in Mins. | Heat Given Off in Small Cals.* | | Mean Temp. of Calorimeters | | Blood-Flow in gm.* | | Pressure in Armlet | |
|---|---|---|---|---|---|---|---|---|
| | R | L | R | L | R | L | R | L |
| 8 | 76.09 | 74.21 | 30.79 | 30.83 | 14.7 | 13.81 | 0 | 0 |
| 10 | 35.52 | 11.64 | 31.50 | 31.35 | ..... | ..... | 65 | 120 |
| 8 | 41.2 | 42.2 | 31.91 | 31.57 | 9.38 | 8.99 | 0 | 0 |

*Per 100 c.c. of hand per minute.

*Experiment 6.*—C. B. Riva-Rocci on right arm, Erlanger on left. Systolic pressure, right arm 110, 106, 100 (average 105); left arm 104, 96, 98 (average 100). At 3:19 p. m. hands put into bath; at 3:30 into calorimeters, 3,015 c.c. water in each. Pulse 106 (sitting); mouth temperature 36.5 C.; rectal 37.0 C. Between 3:40½ and 3:40¾ p. m. pressure raised in armlets to 90 mm. mercury in right, 100 mm. in left. At 3:48 made pressure 80 mm. in right, 110 in left. At 3:53 p. m. made pressure 80 mm. in right, 120 in left. At 3:57 made pressure in left 130, keeping right at 80. At 4:03 p. m. raised pressure in right to 130, keeping left at 130. At 4:10 p. m. released pressure in both armlets. At 4:18 removed hands from calorimeters, dried them and again measured systolic pressure with hands in the same position as during the experiment; right 106, 107; left 108, 112, 104, 108 (average 108). Volume of right hand 430 c.c.; left 424 c.c.

In Experiment 6, from the data of which Figure 6 has been constructed, it is seen that when a pressure equal to the average systolic

## TABLE 9.—CALORIMETER TEMPERATURES DURING EXPERIMENT 6

| Time | Calorimeter Temperature | | | | | Time | Calorimeter Temperature | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Actual | | Corrected | | | | Actual | | Corrected | | |
| | R | L | R | L | Diff | | R | L | R | L | Diff |
| 3.31½ | 29.85 | 29.93 | ..... | ..... | 0.08 | 3.56½ | 30.68 | 30.82 | 30.905 | 31.02 | 0.115 |
| 3.32½ | 29.94 | 30.03 | 29.949 | 30.038 | 0.089 | 3.57½ | 30.69 | 30.815 | 30.924 | 31.023 | 0.099 |
| 3.33½ | 30.01 | 30.11 | 30.028 | 30.126 | 0.098 | 3.58½ | 30.71 | 30.81 | 30.953 | 31.026 | 0.073 |
| 3.34½ | 30.09 | 30.20 | 30.117 | 30.224 | 0.107 | 3.59½ | 30.71 | 30.81 | 30.962 | 31.034 | 0.072 |
| 3.35½ | 30.75 | 30.31 | 30.211 | 30.342 | 0.131 | 4.00½ | 30.72 | 30.81 | 30.981 | 31.042 | 0.061 |
| 3.36 | 30.21 | 30.35 | 30.25 | 30.386 | 0.136 | 4.01½ | 30.735 | 30.805 | 31.005 | 31.045 | 0.040 |
| 3.37 | 30.29 | 30.44 | 30.339 | 30.484 | 0.145 | 4.02½ | 30.75 | 30.805 | 31.029 | 31.053 | 0.024 |
| 3.38 | 30.35 | 30.53 | 30.408 | 30.582 | 0.174 | 4.03½ | 30.755 | 30.805 | 31.043 | 31.061 | 0.018 |
| 3.39 | 30.41 | 30.61 | 30.477 | 30.67 | 0.193 | 4.04½ | 30.77 | 30.80 | 31.067 | 31.064 | 0.003* |
| 3.40 | 30.45 | 30.65 | 30.526 | 30.718 | 0.192 | 4.05½ | 30.78 | 30.805 | 31.086 | 31.077 | 0.009* |
| 3.41½ | 30.50 | 30.72 | 30.59 | 30.80 | 0.210 | 4.06½ | 30.785 | 30.80 | 31.100 | 31.08 | 0.020* |
| 3.42½ | 30.53 | 30.73 | 30.629 | 30.818 | 0.189 | 4.07½ | 30.79 | 30.79 | 31.114 | 31.078 | 0.036* |
| 3.43½ | 30.55 | 30.76 | 30.658 | 30.856 | 0.198 | 4.08½ | 30.785 | 30.79 | 31.118 | 31.086 | 0.032* |
| 3.44½ | 30.57 | 30.78 | 30.687 | 30.884 | 0.197 | 4.09½ | 30.78 | 30.79 | 31.122 | 31.094 | 0.028* |
| 3.45½ | 30.57 | 30.79 | 30.696 | 30.902 | 0.206 | 4.10½ | 30.79 | 30.79 | 31.141 | 31.102 | 0.039* |
| 3.46½ | 30.58 | 30.79 | 30.715 | 30.91 | 0.195 | 4.11½ | 30.81 | 30.83 | 31.170 | 31.150 | 0.020* |
| 3.47½ | 30.575 | 30.79 | 30.719 | 30.918 | 0.199 | 4.12½ | 30.825 | 30.86 | 31.194 | 31.188 | 0.006* |
| 3.48½ | 30.59 | 30.81 | 30.743 | 30.946 | 0.203 | 4.14 | 30.84 | 30.92 | 31.222 | 31.260 | 0.038 |
| 3.49½ | 30.59 | 30.80 | 30.752 | 30.944 | 0.192 | 4.15 | 30.84 | 30.92 | 31.231 | 31.268 | 0.037 |
| 3.50½ | 30.595 | 30.81 | 30.766 | 30.962 | 0.196 | 4.16 | 30.85 | 30.93 | 31.25 | 31.282 | 0.032 |
| 3.51½ | 30.60 | 30.81 | 30.78 | 30.97 | 0.190 | 4.17 | 30.865 | 30.95 | 31.274 | 31.31 | 0.036 |
| 3.52½ | 30.61 | 30.81 | 30.799 | 30.978 | 0.179 | 4.18 | 30.88 | 30.955 | 31.298 | 31.323 | 0.025 |
| 3.53½ | 30.62 | 30.82 | 30.818 | 30.996 | 0.178 | 4.35 | 30.72 | 30.815 | ..... | ..... | ..... |
| 3.54½ | 30.63 | 30.82 | 30.837 | 31.004 | 0.167 | | ..... | ..... | ..... | ..... | .... |
| 3.55½ | 30.65 | 30.82 | 30.866 | 31.012 | 0.146 | | ..... | ..... | ..... | ..... | .... |

*Excess of R. over L. All the other numbers in this column are excesses of L. over R.

pressure was established in the left armlet and a pressure (90 mm.) 15 mm. below average systolic pressure in the right armlet, the curves of heat loss remained practically parallel for the two hands. With 80 mm. in the right armlet, an increase in the heat given off by the right hand. distinct though not great, is shown. With a handicap of 80 mm. mercury, say 25 mm. below the systolic pressure, in the right armlet a sensible although still only a relatively small amount of blood was able to find its way below the cuff, whereas a pressure of 90 mm. in the armlet formed almost a complete barrier.

In Table 10, Experiment 6, it will be observed that with unobstructed circulation the left hand showed an excess of heat given off of 11.28 small calories per 100 c.c. per minute, as compared with the right. The calculated blood-flow (not shown in the table) was 11.45 gm. per 100 c.c. of hand per minute for the right, and 13.46 gm. for the left, an excess of nearly 20 per cent. for the left. This, the opposite of the usual relation between the hands in right-handed persons, is doubtless due to some accidental temporary difference of vasomotor tone in the two hands. The establishment of a pressure of 100 mm. mercury on the left arm, and 90 mm. on the right, completely wiped out the excess of heat loss from

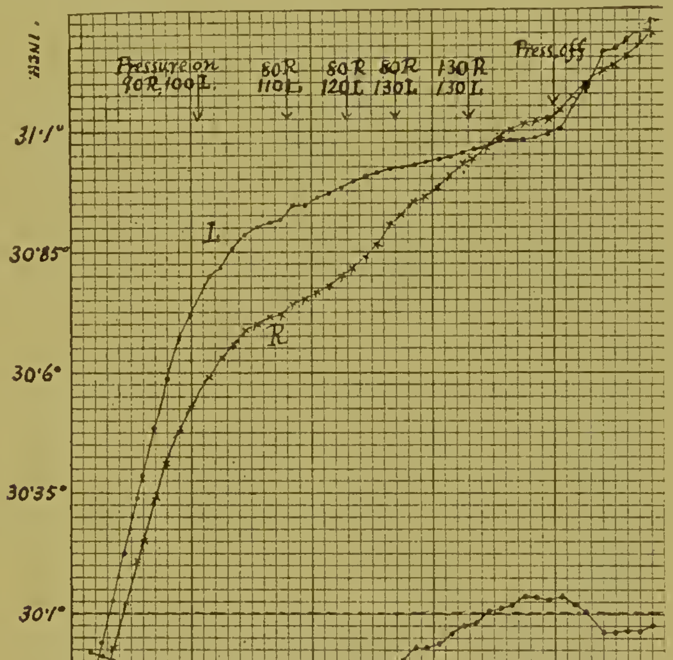

Fig. 6.—R, L and D the same as in the preceding figure but plotted from Experiment 6.

the left hand, converting it into a slight excess for the right. The difference between the two hands with these pressures in the armlets was so slight as to indicate that only a very small part of the heat loss by the right hand could have been due to the circulation of blood in it. When the pressure in the right armlet was reduced to 80 mm. the heat given off by the right hand at once increased, while that given off by the left still continued to fall owing to equalization of the temperature of the left hand and the calorimeter. For the twelve minutes during which the handicap of the right arm was 80 mm., the average amount of heat given off by the right hand per 100 c.c. per minute was, in round numbers, 18 small calories, and by the left 6 small calories. The difference, 12 small calories per 100 c.c. per minute, represents the heat given off by blood flowing through the right hand, amounting to about 20 per cent. of the initial unhindered flow.

TABLE 10.—HEAT GIVEN OFF DURING EXPERIMENT 6

| Period in Mins. | Heat Given Off (Small Cals.) per 100 c.c. Hand per Minute | | | Pressure in Armlet | |
|---|---|---|---|---|---|
| | R | L | Diff. | R | L |
| 8½ | 63.97 | 75.25 | 11.28* | 0 | 0 |
| 6 | 17.32 | 16.19 | 1.13 | 90 | 100 |
| 4 | 14.01 | 6.66 | 7.35 | 80 | 110 |
| 3 | 23.95 | 6.76 | 17.19 | 80 | 120 |
| 5 | 17.58 | 4.86 | 12.72 | 80 | 130 |
| 4 | 7.56 | 3.65 | 3.91 | 130 | 130 |
| 7½ | 17.05 | 24.27 | 7.22* | 0 | 0 |

*Excess of left over right. The other numbers in this column are excesses of right over left.

In the latter part of Experiment 3 (see Experiment 3, Table 5), it is seen that with a pressure of 50 mm. in the armlet, when the systolic pressure was about 115, the handicap was only slight, the heat given off increasing only to a very small extent on releasing the pressure. The change in the right hand, which had been longest subjected to the lower handicap, is taken as a more accurate gauge of the value of the handicap than that in the left, since the alteration of the slope of temperature in the hand following removal of the pressure would be less. The increase on reducing the pressure on the right armlet from 80 to 50 mm. was much greater than when it was reduced from 100 to 80 mm. The flow for the first period of nine minutes, with unobstructed circulation, comes out 11.54 gm. per 100 c.c. per minute for the right, and 11.42 gm. for the left, calculated on the volume of the hands after the swelling had subsided.

For the first period of five minutes after the pressure on the right arm had been reduced to 50 mm., the flow for the right hand was 6.13 gm. and for the second period of five minutes, 6.31 gm. per 100 c.c. per minute. For the next nine minutes it was 6.78 gm., or about 59 per cent. of the initial flow. In reality the handicap caused by a pressure of 50 mm. does not reduce the flow nearly as much as 41 per cent. For the calculated flow for the eight minutes after the pressure was released is only 7.15 gm. per 100 c.c. per minute for the right, and 7.17 gm. for the left. This is only 5 per cent. more than the flow in the right hand with 50 mm. pressure in the armlet. The actual flow, no doubt, would be somewhat greater since a portion of the heat given up by the blood would go to heat the hand. Still, the unhindered flow at this period in the experiment was certainly far below the initial flow, as might be expected after such a prolonged immersion, and, therefore, the reduction caused by 50 mm. pressure in the armlet was almost certainly much nearer to 10 per cent. than to 40 per cent.

In the next series of experiments (Experiments 7, 8 and 9) a pressure equal to or greater than the systolic pressure was maintained in one cuff, while the pressure in the other, originally raised to or above the systolic pressure, was allowed to fall slowly and continuously, the calorimeter temperatures being read from minute to minute. It was thought that by plotting curves showing for each minute the rate of loss of heat for each hand and the pressure in the cuff in which it was allowed to vary, any abrupt change in the blood-flow, as the pressure fell, would be easily detected. The curves reproduced in Figures 7 to 9 (constructed from Tables 11, 12 and 13, Experiments 7 to 9), show that this expectation was fulfilled.

*Experiment 7.*—C. B. Volume of right hand at beginning of experiment 400 c.c., of left 385 c.c. before armlets were put on. A Riva-Rocci armlet 12½ cm. wide was now put on each arm. Systolic pressure right arm 118 mm., left 114 mm. At 3:19½ p. m. hands put into bath after detaching manometers. Room temperature 23.3 C.; mouth temperature 36.3 C.; rectal 37.2 C.; pulse 80 (sitting). At 3:31½ p. m. hands put into calorimeters, 3,015 c.c. water in each. Connected manometers with armlets without getting up any pressure. Between 3:39½ and 3:39¾ p. m. made pressure 120 mm. mercury in each cuff. Kept the pressure steady at 120 in left, allowing that in right to fall slowly. At 4:17 pressure released in both armlets. At 4:27 hands removed from calorimeters. Volume of right hand now 421 c.c., of left 418 c.c., with armlets still on but no pressure Systolic and diastolic pressures were now determined by the Erlanger sphygmomanometer. Right arm, systolic 118, diastolic 78; systolic 118 and diastolic 78 in two observations. Left arm systolic 122, diastolic 78. At 5:03 p. m., after armlets were taken off, volume of right hand 402 c.c., of left 395 c.c.

*Experiment 8.*—M. C. Riva-Rocci broad armlets on both arms. Systolic pressure: right 122, 124; left 126, 124 in two observations. Volume of hands with armlets on but loose and with no pressure, right 477 c.c., left 455 c.c. Hands put into bath at 2:33½ p. m. Mouth temperature 36.8 C.; rectal 37.5 C.; room 21.4 C.; pulse 100 (sitting). At 2:45½ p. m. hands put into calorimeters, 3,015 c.c. water in each. Between 2:54½ and 2:54¾ p. m. pressure raised to 130 mm. mercury in each armlet. Maintained this in left while allowing right to fall

TABLE 11.—RECORD OF CALORIMETER TEMPERATURES UNDER VARYING PRESSURES IN EXPERIMENT 7

| Time | Calorimeter Temperature | | | | | Pressure in Right Armlet | Time | Calorimeter Temperature | | | | | Pressure in Right Armlet |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Actual | | Corrected | | | | | Actual | | Corrected | | | |
| | R | L | R | L | Diff. | | | R | L | R | L | Diff. | |
| 3.33 | 30.35 | 30.29 | ...... | ...... | 0.06 | ... | 3.58 | 30.575 | 30.525 | 30.847 | 30.777 | 0.070 | 57 |
| 3.34 | 30.39 | 30.33 | 30.401 | 30.341 | 0.06 | ... | 3.59 | 30.58 | 30.52 | 30.863 | 30.783 | 0.080 | 56 |
| 3.35 | 30.41 | 30.36 | 30.432 | 30.381 | 0.051 | ... | 4.00 | 30.585 | 30.515 | 30.879 | 30.788 | 0.091 | 55 |
| 3.36 | 30.44 | 30.40 | 30.473 | 30.432 | 0.041 | ... | 4.02 | 30.595 | 30.505 | 30.911 | 30.794 | 0.097 | 54 |
| 3.37 | 30.485 | 30.46 | 30.529 | 30.502 | 0.027 | ... | 4.04 | 30.60 | 30.495 | 30.938 | 30.800 | 0.138 | 53 |
| 3.38 | 30.50 | 30.48 | 30.555 | 30.533 | 0.022 | ... | 4.06 | 30.61 | 30.48 | 30.970 | 30.801 | 0.169 | 51.5 |
| 3.39 | 30.515 | 30.51 | 30.580 | 30.573 | 0.007 | 0 | 4.08 | 30.625 | 30.465 | 31.006 | 30.801 | 0.205 | 50 |
| 3.40½ | 30.53 | 30.53 | 30.612 | 30.609 | 0.003 | 112 | 4.10 | 30.665 | 30.44 | 31.068 | 30.792 | 0.276 | 48 |
| 3.41½ | 30.535 | 30.535 | 30.628 | 30.624 | 0.004 | 106 | 4.11 | 30.675 | 30.435 | 31.089 | 30.797 | 0.292 | 47 |
| 3.42½ | 30.535 | 30.535 | 30.639 | 30.635 | 0.004 | 101 | 4.12 | 30.68 | 30.43 | 31.105 | 30.803 | 0.302 | 45 |
| 3.43½ | 30.54 | 30.535 | 30.654 | 30.645 | 0.009 | 97 | 4.13 | 30.69 | 30.43 | 31.126 | 30.813 | 0.313 | 44 |
| 3.44½ | 30.545 | 30.54 | 30.670 | 30.651 | 0.019 | 92.5 | 4.14 | 30.69 | 30.43 | 31.137 | 30.824 | 0.313 | 43 |
| 3.45½ | 30.55 | 30.545 | 30.686 | 30.666 | 0.020 | 88 | 4.15 | 30.69 | 30.425 | 31.148 | 30.829 | 0.319 | 42 |
| 3.46½ | 30.55 | 30.545 | 30.697 | 30.677 | 0.020 | 86 | 4.16 | 30.695 | 30.42 | 31.164 | 30.835 | 0.329 | 40 |
| 3.47½ | 30.55 | 30.545 | 30.708 | 30.687 | 0.021 | 81 | 4.17 | 30.695 | 30.415 | 31.175 | 30.840 | 0.335 | 0 |
| 3.48½ | 30.55 | 30.55 | 30.719 | 30.703 | 0.016 | 78 | 4.18 | 30.695 | 30.43 | 31.185 | 30.866 | 0.319 | ... |
| 3.49½ | 30.55 | 30.55 | 30.730 | 30.713 | 0.017 | 75 | 4.19 | 30.697 | 30.46 | 31.198 | 30.906 | 0.292 | ... |
| 3.50½ | 30.545 | 30.545 | 30.736 | 30.719 | 0.017 | 72 | 4.21 | 30.697 | 30.53 | 31.220 | 30.993 | 0.227 | ... |
| 3.51½ | 30.55 | 30.54 | 30.752 | 30.724 | 0.028 | 69 | 4.22 | 30.697 | 30.54 | 31.231 | 31.013 | 0.218 | ... |
| 3.52½ | 30.55 | 30.535 | 30.763 | 30.730 | 0.033 | 67 | 4.23 | 30.695 | 30.55 | 31.240 | 31.034 | 0.206 | ... |
| 3.54 | 30.555 | 30.53 | 30.784 | 30.740 | 0.044 | 63 | 4.24 | 30.69 | 40.56 | 31.246 | 31.054 | 0.192 | ... |
| 3.55 | 30.56 | 30.53 | 30.800 | 30.751 | 0.049 | 61 | 4.25 | 30.695 | 30.565 | 31.262 | 31.070 | 0.192 | ... |
| 3.56 | 30.565 | 30.53 | 30.816 | 30.761 | 0.055 | 59 | 4.26 | 30.69 | 30.58 | 31.268 | 31.095 | 0.173 | ... |
| 3.57 | 30.57 | 30.525 | 30.832 | 30.767 | 0.065 | 58 | 4.27 | 30.69 | 30.595 | 31.279 | 31.121 | 0.158 | ... |
| | ...... | ...... | ...... | ...... | ..... | .... | 4.57 | 30.36 | 30.28 | ...... | ...... | ..... | ... |
| | ...... | ...... | ...... | ...... | ..... | .... | 5.00 | 30.33 | 30.26 | ...... | ...... | ..... | ... |

slowly. At 3:19½ p. m. pressure released in both. At 3:29 p. m. hands taken out of calorimeters. Volume of hands again measured (with armlets off); right 496 c.c., left 490 c.c. Replaced armlets and measured systolic and diastolic pressures with Erlanger apparatus. Right: Systolic 122, diastolic 88; another observation. diastolic 90; another diastolic 86; another observation, systolic 120, diastolic 88. Left arm: Systolic 124, diastolic 94; another observation, systolic 128, diastolic 90. Repeated measurement on right arm: diastolic 90, systolic 126, diastolic 82. As always, the hands were hanging down as if in the calorimeters, subject seated.

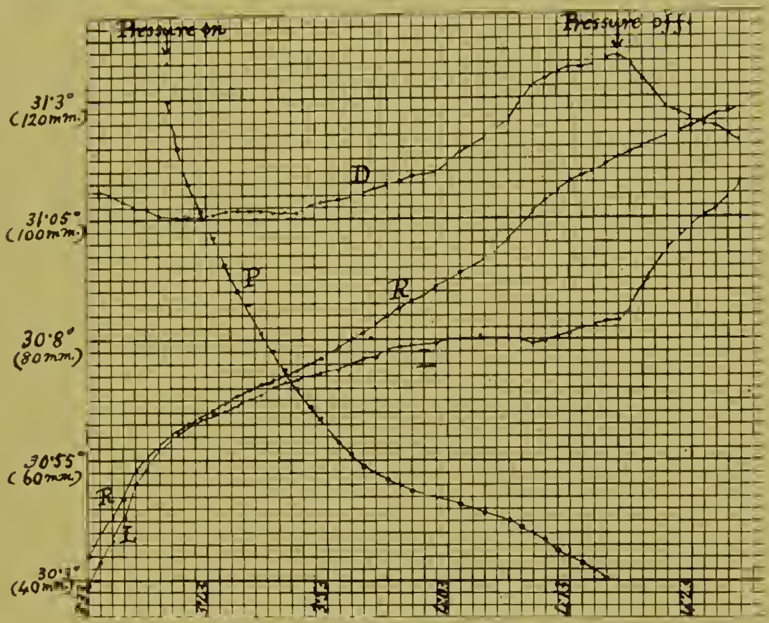

Fig. 7.—R and L (corrected) calorimeter temperatures for right and left hands, respectively; D, differences between these temperatures; P, curve showing fall of pressure in right armlet. Plotted from Experiment 7.

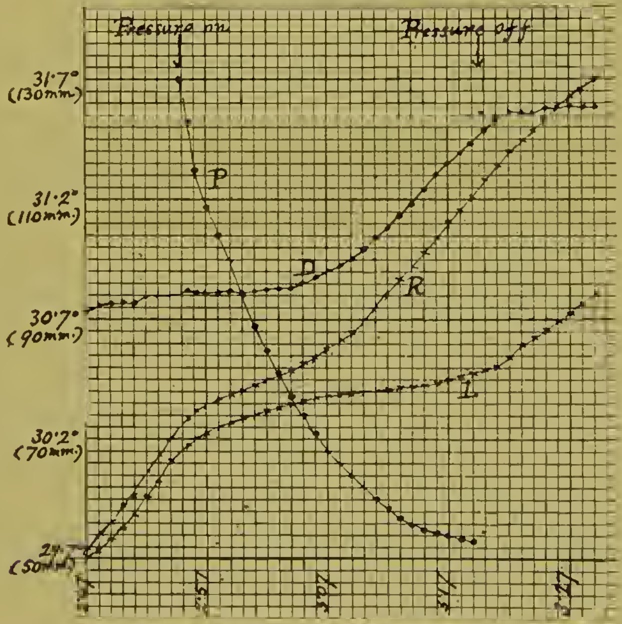

Fig. 8.—The letters have the same signification as in Fig. 7, but the curves are plotted from Experiment 8.

### TABLE 12.—RECORD OF CALORIMETER TEMPERATURES, EXPERIMENT 8

| Time | Calorimeter Temperature | | | | | Pressure in right Armlet |
|---|---|---|---|---|---|---|
| | Actual | | Corrected | | | |
| | R | L | R | L | Diff.* | |
| 2.47 | 29.74 | 29.705 | ...... | ...... | 0.035 | ... |
| 2.48 | 29.79 | 29.73 | 29.805 | 29.744 | 0.061 | ... |
| 2.49 | 29.825 | 29.755 | 29.855 | 29.783 | 0.072 | ... |
| 2.50 | 29.87 | 29.795 | 29.915 | 29.837 | 0.078 | ... |
| 2.51 | 29.90 | 29.84 | 29.96 | 29.896 | 0.064 | ... |
| 2.52 | 29.98 | 29.89 | 30.065 | 29.960 | 0.105 | ... |
| 2.53 | 30.05 | 29.95 | 30.14 | 30.034 | 0.106 | ... |
| 2.54 | 30.10 | 30.01 | 30.205 | 30.108 | 0.097 | 0 |
| 2.55½ | 30.17 | 30.06 | 30.297 | 30.179 | 0.118 | 123 |
| 2.56 | 30.18 | 30.075 | 30.315 | 30.201 | 0.114 | 115 |
| 2.57 | 30.195 | 30.09 | 30.345 | 30.230 | 0.115 | 109 |
| 2.58 | 30.205 | 30.10 | 30.370 | 30.254 | 0.116 | 104 |
| 2.59 | 30.21 | 30.10 | 30.390 | 30.268 | 0.122 | 100 |
| 3.00 | 30.21 | 30.11 | 30.405 | 30.292 | 0.113 | 94 |
| 3.01 | 30.215 | 30.11 | 30.425 | 30.306 | 0.119 | 89 |
| 3.02 | 30.22 | 30.11 | 30.445 | 30.320 | 0.125 | 85 |
| 3.03 | 30.225 | 30.11 | 30.465 | 30.334 | 0.131 | 81 |
| 3.04 | 30.23 | 30.105 | 30.485 | 30.343 | 0.142 | 77.5 |
| 3.05 | 30.25 | 30.11 | 30.520 | 30.362 | 0.158 | 74 |
| 3.06 | 30.275 | 30.11 | 30.560 | 30.376 | 0.184 | 71 |
| 3.07 | 30.29 | 30.10 | 30.590 | 30.380 | 0.210 | 68 |
| 3.08 | 30.297 | 30.09 | 30.612 | 30.384 | 0.228 | 66 |
| 3.09 | 30.315 | 30.085 | 30.645 | 30.393 | 0.252 | 64 |
| 3.10 | 30.35 | 30.08 | 30.695 | 30.402 | 0.293 | 62 |
| 3.11 | 30.395 | 30.07 | 30.755 | 30.406 | 0.349 | 60 |
| 3.12 | 30.425 | 30.065 | 30.80 | 30.415 | 0.385 | 58.5 |
| 3.13 | 30.475 | 30.06 | 30.865 | 30.424 | 0.441 | 57 |
| 3.14 | 30.51 | 30.05 | 30.915 | 30.428 | 0.487 | 56 |
| 3.15 | 30.565 | 30.045 | 30.985 | 30.437 | 0.548 | 55 |
| 3.16 | 30.61 | 30.035 | 31.045 | 30.441 | 0.604 | 54.5 |
| 3.17 | 30.655 | 30.035 | 31.105 | 30.455 | 0.650 | 54 |
| 3.18 | 30.695 | 30.03 | 31.160 | 30.464 | 0.696 | 53.5 |
| 3.19 | 30.73 | 30.03 | 31.210 | 30.478 | 0.732 | 53 |
| 3.20 | 30.79 | 30.03 | 31.285 | 30.492 | 0.793 | 0 |
| 3.21 | 30.83 | 30.035 | 31.340 | 30.511 | 0.829 | ... |
| 3.22 | 30.88 | 30.055 | 31.405 | 30.545 | 0.860 | ... |
| 3.23 | 30.905 | 30.09 | 31.445 | 30.594 | 0.851 | ... |
| 3.24 | 30.925 | 30.11 | 31.480 | 30.628 | 0.852 | ... |
| 3.25 | 30.97 | 30.125 | 31.540 | 30.657 | 0.883 | ... |
| 3.26 | 30.995 | 30.15 | 31.580 | 30.696 | 0.884 | ... |
| 3.27 | 31.02 | 30.165 | 31.62 | 30.725 | 0.895 | ... |
| 3.28 | 31.04 | 30.19 | 31.655 | 30.764 | 0.891 | ... |
| 3.29 | 31.07 | 30.22 | 31.70 | 30.808 | 0.892 | ... |
| | ...... | ...... | ...... | ...... | ..... | .... |

*Excess of R. over L.

*Experiment 9.*—M. C. 2:45 p. m. volume of hands before armlets were put on, right 450 c.c., left 444 c.c. At 3:00 p. m. blood-pressure determined with Riva-Rocci apparatus by stethoscope method. Right arm: At 122 sound began; at 92 began to decrease; at 82 disappeared completely. Another observation: At 95 sound began to decrease; at 82 completely gone. Another observation: At 124 sound began; at 98 became suddenly less; at 85 completely gone. At 3:13½ p. m. hands put into bath with armlets on but no pressure. Mouth temperature 36.7 C., rectal 37.05 C., pulse (sitting) 74. At 3:33½ p. m. hands put into calorimeters. Between 3:43 5/6 and 3:44 p. m. established a pressure of 130 mm. in left and 140 in right armlet (broad Riva-Rocci). Maintained pressure at 130 in left, and let it slowly sink in right. At 4:05½ p. m. released pressure in both. At 4:11½ p. m. hands taken out of calorimeters and dried. Blood-pressure again determined in right arm by Riva-Rocci with stethoscope, and with Erlanger method and stethoscope simultaneously. At 122 sound began to be heard (this was also the systolic pressure by Erlanger record); at 98 sound suddenly began to diminish; reappeared at 92, though not as loud as at first. At 88 sound finally disappeared, and this corresponded to the diastolic pressure by Erlanger record. Other observations gave 85, 86 and 92 as diastolic pressure by stethoscope method. Volume of hands again measured after taking off armlets; right 486 c.c. left 461 c.c.

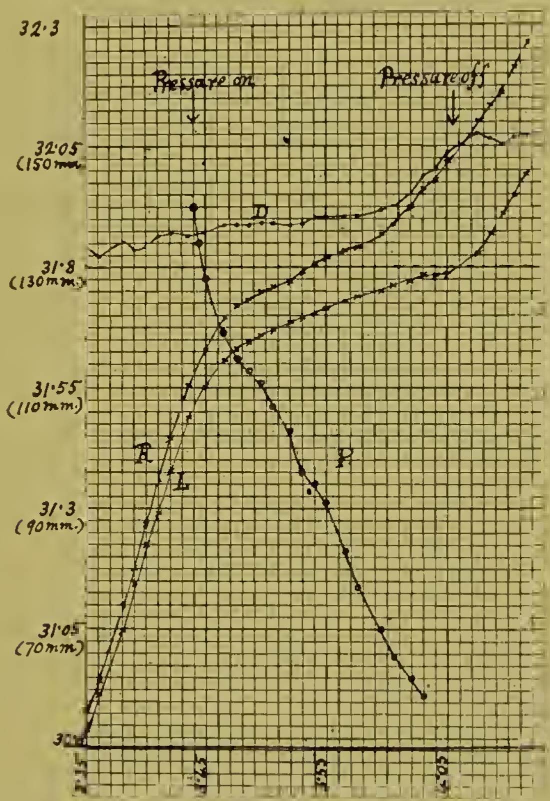

Fig. 9.—The letters have the same signification as in Figs. 7 and 8, but the curves are plotted from Experiment 9.

It will be seen at a glance, in Figures 7, 8 and 9, that as the pressure goes on diminishing in one armlet from the systolic pressure, the curves R and L, representing the rate of heat loss of the two hands to the calorimeters, remain practically parallel till the pressure has fallen very

TABLE 13.—RECORD OF CALORIMETER TEMPERATURES, EXPERIMENT 9

| Time | Calorimeter Temperature | | | | | Pressure on Right Arm |
|---|---|---|---|---|---|---|
| | Actual | | Corrected | | | |
| | R | L | R | L | Diff. | |
| 3.35 | 30.88 | 30.84 | ...... | ...... | 0.04 | ... |
| 3.36 | 30.93 | 30.91 | 30.94 | 30.919 | 0.021 | ... |
| 3.38 | 31.07 | 31.02 | 31.10 | 31.048 | 0.052 | ... |
| 3.39 | 31.14 | 31.11 | 31.18 | 31.147 | 0.033 | ... |
| 3.40 | 31.22 | 31.18 | 31.27 | 31.226 | 0.044 | ... |
| 3.41 | 31.295 | 31.235 | 31.355 | 31.290 | 0.065 | ... |
| 3.42 | 31.375 | 31.31 | 31.445 | 31.374 | 0.071 | ... |
| 3.43½ | 31.47 | 31.41 | 31.555 | 31.488 | 0.067 | 0 |
| 3.45 | 31.525 | 31.46 | 31.625 | 31.552 | 0.073 | 128 |
| 3.46½ | 31.58 | 31.50 | 31.695 | 31.606 | 0.089 | 119 |
| 3.47½ | 31.595 | 31.515 | 31.72 | 31.630 | 0.090 | 115 |
| 3.48½ | 31.595 | 31.52 | 31.73 | 31.644 | 0.086 | 113 |
| 3.49½ | 31.605 | 31.525 | 31.75 | 31.658 | 0.092 | 111 |
| 3.50½ | 31.605 | 31.525 | 31.76 | 31.667 | 0.093 | 107 |
| 3.52 | 31.60 | 31.53 | 31.77 | 31.686 | 0.084 | 103 |
| 3.53 | 31.607 | 31.53 | 31.787 | 31.695 | 0.092 | 96 |
| 3.54 | 31.615 | 31.53 | 31.805 | 31.704 | 0.101 | 94 |
| 3.55 | 31.62 | 31.53 | 31.82 | 31.714 | 0.106 | 91 |
| 3.56½ | 31.62 | 31.53 | 31.835 | 31.728 | 0.107 | 83 |
| 3.57½ | 31.615 | 31.53 | 31.84 | 31.737 | 0.103 | 77 |
| 3.59½ | 31.625 | 31.525 | 31.87 | 31.750 | 0.120 | 70 |
| 4.00½ | 31.635 | 31.53 | 31.89 | 31.764 | 0.126 | 65.5 |
| 4.02 | 31.655 | 31.525 | 31.925 | 21.773 | 0.152 | 62 |
| 4.03 | 31.685 | 31.525 | 31.965 | 31.782 | 0.183 | 59 |
| 4.04 | 31.69 | 31.515 | 31.98 | 31.781 | 0.199 | ... |
| 4.05 | 31.72 | 31.515 | 32.02 | 31.791 | 0.229 | ... |
| 4.07½ | 31.78 | 31.53 | 32.105 | 31.829 | 0.276 | 0 |
| 4.08½ | 31.80 | 31.565 | 32.135 | 31.873 | 0.262 | ... |
| 4.09½ | 31.825 | 31.595 | 32.165 | 31.912 | 0.253 | ... |
| 4.10½ | 31.86 | 31.62 | 32.215 | 31.946 | 0.269 | ... |
| 4.11½ | 31.90 | 31.655 | 32.265 | 31.991 | 0.274 | ... |
| 4.52 | 31.50 | 31.29 | ...... | ...... | ..... | ... |

considerably below the systolic pressure. This is shown most clearly in the curve D, which depicts the difference in temperature of the two calorimeters at each point. For a time it remains approximately parallel to the abscissa axis. The first abrupt change in D, obviously indicating the point at which curves R and L begin to diverge sharply, corresponds to a pressure of 72 mm. in Figure 7 (systolic pressure 118 mm.), 77.5 mm. in Figure 8 (systolic pressure 122 mm.), and 77 mm. in Fig. 9 (systolic pressure 123 mm).

In Tables 14, 15 and 16 from the same three experiments the quantities of heat given off by the two hands are compared for the different pressures by taking the average for periods comprising several minutes. Here also it will be noticed that the differences in the heat loss of the two hands experience the first really marked increase when the pressure in the right armlet is falling, in Experiment 7 from 67 to 61 mm., in Experiment 8 from 77.5 to 64 mm., and in Experiment 9 from 77 to 65.5 mm.

The pressures at which the blood-flow is first notably increased in the observations in which the diminution of pressure in the armlet was produced abruptly by successive considerable decrements, agree very well with the "critical" pressures obtained by the method of continuous escape. The same is true of the critical pressure determined by raising the pressure in the armlet till the flow first begins to be seriously handicapped.

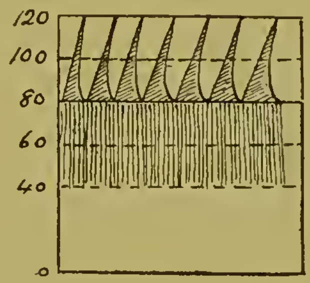

Fig. 10.—For description see text.

It is easily understood why for some distance below the systolic pressure the increase in blood-flow should be inconspicuous. Only the tops of the systolic waves are finding their way beyond the obstruction of the cuff, and the total flow in a given time is measured by the united areas inclosed between the tops of the curve and the horizontal lines representing the pressure in the cuff. Thus in the diagram (Fig. 10) the increase in the flow when the pressure is reduced from the systolic pressure, 120, to 100, is obviously slight. Even with a diminution to 80 the combined areas represent less than half of the area of the rectangle formed by the lines 120 and 80 and the perpendiculars joining them. A drop of 40 mm. in the armlet from 80 to 40 will clearly produce a much greater increase in the flow than a drop of 40 mm. from 120 to 80. This is on the assumption that the lumen of the arteries increases equally for equal decrements

TABLE 14.—HEAT GIVEN OFF BY HANDS IN EXPERIMENT 7

| Period in Minutes | Heat Given Off (Small Cal.) per 100 c.c. Hand per Minute | | | Pressure in Armlet | |
|---|---|---|---|---|---|
| | R | L | Diff. | R | L |
| 6 | 32.70 | 40.70 | 8.00* | 0 | 0 |
| 5 | 12.80 | 11.48 | 1.32 | 120- 88 | 120 |
| 7 | 9.25 | 7.57 | 1.68 | 88- 67 | 120 |
| 2½ | 12.60 | 7.06 | 5.54 | 67- 61 | 120 |
| 5 | 13.35 | 6.18 | 7.17 | 61- 55 | 120 |
| 8 | 13.56 | 3.31 | 10.25 | 55- 50 | 120 |
| 8 | 16.44 | 3.86 | 12.58 | 50- 40 | 120 |
| 10 | 8.95 | 24.43 | 15.48* | 0 | 0 |

*Excess of left over right. The other numbers in this column are excesses of right over left.

TABLE 15.—HEAT GIVEN OFF BY HANDS IN EXPERIMENT 8

| Period in Minutes | Heat Given Off (Small Cal.) per 100 c.c. Hand per Minute | | | Pressure in Armlet | |
|---|---|---|---|---|---|
| | R | L | Diff.* | R | L |
| 7 | 48.43 | 42.57 | 5.86 | 0 | 0 |
| 3½ | 19.17 | 18.21 | 0.96 | 123-100 | 130 |
| 5 | 13.42 | 10.19 | 3.23 | 100- 77.5 | 130 |
| 5 | 23.31 | 6.37 | 16.94 | 77.5- 64 | 130 |
| 2 | 40.04 | 3.84 | 36.20 | 64- 60 | 130 |
| 4 | 41.93 | 4.78 | 37.15 | 60- 55 | 130 |
| 4 | 40.98 | 6.64 | 34.34 | 55- 53 | 130 |
| 5 | 37.19 | 23.87 | 13.32 | 0 | 0 |
| 4 | 29.14 | 27.58 | 1.56 | 0 | 0 |

*Excess of right over left.

TABLE 16.—HEAT GIVEN OFF BY HANDS IN EXPERIMENT 9

| Period in Minutes | Heat Given Off (Small Cal.) per 100 c.c. Hand per Minute | | | Pressure in Armlet | |
|---|---|---|---|---|---|
| | R | L | Diff. | R | L |
| 8½ | 60.52 | 58.95 | 1.57 | 0 | 0 |
| 3* | 35.85 | 31.53 | 4.32 | 140-119 | 130 |
| 5½ | 10.34 | 11.18 | 0.84† | 119-103 | 130 |
| 5½ | 9.74 | 7.04 | 2.70 | 103- 77 | 130 |
| 3 | 12.74 | 6.98 | 5.76 | 77- 65.5 | 130 |
| 2½ | 23.02 | 5.40 | 17.62 | 65.5- 59 | 130 |
| 4 | 30.72 | 31.08 | 0.36† | 0 | 0 |

*Pressure established in armlets only for the last 2½ minutes of this period.

†Excess of left over right; the other numbers in this column are excesses of right over left.

of external pressure, i. e., for equal increments in excess of internal over external pressure, within the whole range of the pressures in question. But MacWilliam[2] has shown that for excised arteries not in a state of continuous post-mortem contraction, the expansion when pressure is applied within the lumen is much greater in proportion to the pressure for the first increments of pressure than for further increments. If we can assume that the arteries in the living arm are "soft" in the sense in which MacWilliam uses the term in the case of excised arteries relaxed by potassium sulphocyanid, or by keeping in blood at body temperature for twenty-four hours, we have an easy explanation of the fact that after the critical point has been reached, removal of the first 40 or 50 mm. pressure in the cuff produces a far greater increase in the blood-flow than removal of the second 40 or 50 mm. Suppose the diastolic pressure is 90 mm. Then when the pressure in the cuff is lowered from 90 to 70 mm. the arteries are distended by a permanent excess of pressure of 20 mm. A further diminution of pressure in the cuff of 20 mm., i. e., to 50 mm., corresponds to a permanent distending pressure of 40 mm. within the lumen of the artery. A further diminution of 20 mm. in the armlet (i. e., to 30 mm.), corresponds to a distending pressure in the arteries of 60 mm. In one experiment of MacWilliam the increase in cubic capacity of the artery (ox carotid) produced by increasing the internal pressure from 0 to 20 mm. mercury was 14 (in arbitrary units); from 20 to 40, 13; i. e., in all 27 for the first increment of 40 mm.; while an increment of pressure from 40 to 60 mm. caused an increase in cubic capacity of only 9, and from 60 to 80 mm. an increase of only 8, or 17 in all for the second increment of 40 mm. in the pressure. The initial capacity of the piece of artery (17 mm. long) would be 330 c.mm., the lumen being 5 mm. in diameter with zero pressure. From the data given, which, however, are not quite complete for such a calculation, it may be reckoned that after the application of the first 40 mm. pressure the capacity was increased to 520 c.mm. and the diameter to 6.3 mm., while after the second increment of 40 mm. pressure the capacity was 640 c.mm. and the diameter 6.9 mm. This is on the assumption that the capacity was increased mainly by an increase in the mean cross-section of the segment of artery, without appreciable increase in its length, an assumption at any rate very likely correct for living arteries like the brachial opening up under the influence of the internal pressure as the pressure in the armlet falls. Now by Poiseuille's law for tubes beyond the capillary size the volume of outflow varies as the cube of the diameter; i. e., in the experiment in question the flows for the three conditions of the artery would be in the ratio $5^3 : (6{\cdot}3)^3 : (6{\cdot}9)^3$ or 1 : 2 : 2·6. The second increment of 40 mm. would therefore produce little more than half as great an increase in the flow as the first increment of 40 mm. in the pressure, on the supposition that the flow through the arteries in the region of the block depends merely on the cross-section of the arteries and varies with the cross-section according to Poiseuille's law. In our observations the difference would be much greater, for in MacWilliam's experiment the artery possessed so much rigidity that before pressure was established the lumen had a diameter of 5 mm., while in the living arm with a pressure in the cuff not less than the diastolic pressure, it can be assumed that they collapse completely after each pulsation.

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[2] MacWilliam: Proc. Roy. Soc., 1901, lxx, 109.

So far we have not taken into account the complicating factor that not only the arteries but the veins of the upper arm are compressed by the cuff. It may be asked how do we know that the total block which we have demonstrated to occur with systolic pressure in the armlet is due to complete obliteration of the arteries and not simply to obliteration of the veins while the arteries still remain partially open? The answer to this is that the conclusions do not depend on observations made immediately after the establishment of the various pressures in the armlets. If the arteries remain open with any given pressure and the veins are occluded, the pressure in the veins must eventually become equal to the arterial pressure, and blood will then again escape from the veins. The handicap of the cuff, in so far as it consists in compression of the veins, will thus be overcome, and the flow, the veins having once been filled to their new capacity, will then again become steady, the same quantity of blood leaving the arm by the veins as enters it by the arteries. As a matter of fact, Hill and Flack[3] have shown by actual experiment that when a pressure slightly below the systolic pressure is applied to the upper arm, the venous pressure in the forearm becomes equal to that in the armlet. In a man with a systolic pressure of 220 mm., Hill applied a pressure of 200 mm. to the arm and measured a venous pressure of 200 mm. in the forearm. He did not approach nearer to the systolic pressure because of the discomfort caused to the patient.

In the observations which we have been discussing above it will be noted that the critical point in the falling armlet pressure, below which a further diminution of pressure is accompanied by a sharp increase in the flow, does not coincide with the diastolic pressure as measured by any of the clinical methods used but with a pressure usually about 10 mm. lower. In other words, the pressure in the cuff can be diminished 10 mm. below the "diastolic" pressure without apparently rendering it sensibly easier for blood to pass through the previously compressed arteries. A further diminution of the pressure in the armlet by 10 mm., however, leads to a distinct increase. The most obvious suggestion is that the clinical methods of measuring diastolic pressure give somewhat too high a result. But it is possible that as our experiments necessarily involve a considerably longer compression of the arm than is practiced in clinical measurements of blood-pressure, the rigidity of the blood-vessels themselves or of the tissues immediately surrounding them, when the pressure begins to be reduced, is greater than normal, owing perhaps to increased tonic contraction of the vascular walls or edema of the tissues. But the discussion of this question would lead us too far, especially as it is not necessary for the purposes of the present paper. New data would also be required before it could be profitably considered.

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[3] Hill and Flack: Brit. Med. Jour., 1909, i, 272; Proc. Physiol. Soc., Feb. 27, 1909; Jour. Physiol., 1909, xxxviii, 49. Hill, Heart, 1909, i, 74.

Certain practical applications of the observations described suggest themselves. It is probable, e. g., that in pathological cases measurements such as those given in Section II might throw light on the condition of the vessels as regards extensibility. The response of the blood flow in the hands to handicaps of various amounts (below the diastolic pressure) applied to the arm might even be expected to give something of quantitative precision to such investigations. In conditions of arterial hypertonus due to increased peripheral resistance a given handicap on the arm might be expected to produce a smaller relative diminution in the flow through the hand than with normal arterial pressure or in conditions of increased arterial pressure due to increased cardiac action, if such exist. In certain cardiac lesions also it would seem that results of practical interest might be obtained by comparing the change in the flow through the hand produced by a given handicap on the arm with the effect produced in normal individuals. In aortic insufficiency, for instance, it is to be expected that a greater diminution of pressure from the systolic pressure would be required to cause a sensible increase in the amount of heat given off by the hand than is normally the case, and the difference might afford some index of the degree of insufficiency. Finally, the ease or difficulty, as tested by the volume of the circulation in the hand with which a given handicap is borne at different periods in the history of one and the same case, might afford information supplementary to that acquired by other methods, of progress or the reverse. By the use of the foot calorimeters the method is, of course, equally applicable to the lower extremities.

## SUMMARY

### SECTION I

It is concluded, on the basis of observations on two healthy young men, that a pressure equal to the systolic pressure, as estimated by the clinical methods used, applied to the upper arm by a broad cuff, causes complete cessation of the blood flow in the hand. Since the pressure in the veins distal to the constricting armlet eventually becomes equal to the systolic arterial pressure, this is taken to mean that the lumen of the arteries under the cuff is actually obliterated.

### SECTION II

1. When the pressure in an armlet compressing the upper arm is reduced from the systolic arterial pressure the blood flow in the corresponding hand is only slightly increased for a considerable decrement of pressure.

2. The pressure in the armlet must fall somewhat below the "diastolic" pressure, as clinically determined, before any marked increase in the flow through the hand occurs.

3. The first decrements of pressure below this "critical" pressure are accompanied by a much greater increase in the flow through the hand than further equal decrements.

4. A handicap of half the "diastolic" pressure causes only a relatively small diminution in the flow through the hand.

5. It is suggested that the method of handicapping the circulation in the arm or leg by known pressures and observing in what degree the handicap is overcome, may in certain cases constitute a useful supplementary method of clinical investigation.

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# Einfluß der Herztemperatur auf die Tätigkeit der Hemmungsnerven des Herzens.

Von

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(Mit 14 Textfiguren.)

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Vor 20 Jahren[1]) unternahm ich eine Untersuchung, die sich u. a. auch mit der Frage beschäftigte, wieweit die Einwirkung der Hemmungs- und Beschleunigungsnerven auf das Herz geändert werden könnte durch Beeinflussung des physiologischen Zustandes vom Herzens selbst. Es war zu hoffen, daß auf diesem Wege etwas Licht in die Natur der Hemmung und Beschleunigung der Herztätigkeit gebracht werden könnte. Änderungen im Endokardialdruck und besonders der Temperatur des Herzens wurden als Mittel zur Veränderung seines physiologischen Zustandes gewählt. Der Temperaturwechsel wurde möglichst auf das Herz lokalisiert, während die Extrakardialnerven auf Zimmertemperatur gehalten wurden. In Versuchen, die hauptsächlich am Frosch (Rana temporaria) ausgeführt wurden, kam ich zu der Schlußfolgerung, daß sich die Vaguswirksamkeit vermindert, wenn das Herz unter die gewöhnliche Temperatur (im allgemeinen 11—16°) abgekühlt wird, aber zunimmt, sobald das Herz über diese Temperatur gebracht wird. Luchsinger und J. M. Ludwig[1]) stellten 10 Jahre früher auf Grund ihrer Versuche mittels einfacher Beobachtung an Fröschen und Schildkröten folgendes fest: »Nicht nur bleibt der N. vagus selbst in den höchsten, überhaupt zuträglichen Temperaturen noch vollkommen wirksam und vermag sogar das kaum sich erholende Herz sofort wieder stillzustellen, ja er scheint in diesen letalen Temperaturen an Wirksamkeit eher noch gewonnen zu haben.«

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[1]) Journ. of Physiology 1892, Bd. 13, S. 59.

Ich fand den Vagus noch an der Grenze des Wärmestillstandes wirksam.

Seit dieser Zeit ist eine Anzahl Anhandlungen über den Einfluß der Temperatur auf den Hemmungsmechanismus erschienen, auch eine von Pretschistenskaja[2]), in der er sehr entgegenkommend gewisse Unterschiede zwischen seinen Resultaten und den meinigen feststellt und den mutmaßlichen Grund für diese Unterschiede bespricht. Meine Aufmerksamkeit wurde erst kürzlich auf diese Arbeit gelenkt, welche mir bei ihrer Veröffentlichung durch Zufall entgangen war. Es gibt nur wenig Probleme, bei denen die Schlußfolgerungen verschiedener Beobachter so weit auseinandergehen. Dies rührt meiner Ansicht nach von verschiedenen Ursachen her:

1. Die zur Prüfung der Wirksamkeit für die Hemmungsnerven des Herzens benutzten Kriterien waren nicht immer die gleichen.

Die einen Forscher benutzen den Grenzwert der Reizintensität, der völligen Stillstand herbeiführt, andere die Zeitdauer des bei einer bestimmten Reizstärke hervorgerufenen Stillstandes, wieder andere betrachten die Verlangsamung des Herzschlages, ohne daß ein absoluter Stillstand erzeugt wurde, als entscheidendes Merkmal. Es würde nicht schwer sein, Beispiele dafür anzuführen, wo durch Mangel an scharfer Unterscheidung der verschiedenen Kriterien Unklarheit verursacht wurde.

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[1]) Arch. f. d. ges. Physiol. 1881, Bd. 25 S. 211.

[2]) Zeitschr. f. Biol. 1906, Bd. 47 S. 97.

Vor längerer Zeit habe ich darauf hingewiesen, daß es zwar bei höherer Temperatur leichter sein könne, Hemmung zu erzielen, diese aber dann nicht so lang andauern dürfte als vielleicht bei niederer Temperatur. Daraus könnte man gerechtfertigterweise den Schluß ziehen, daß die Erregbarkeit des Vagus bei höherer Herztemperatur gesteigert ist, wenn der zum Eintritt der Hemmung nötige Reizgrenzwert dafür allein als Kriterium benutzt würde. Anderseits wäre aber diese Schlußfolgerung ungenau, wenn man die Dauer der Hemmungswirkung als unterscheidendes Merkmal gelten ließe. Ich selbst habe in der oben erwähnten Abhandlung betont, wie wichtig es sei, sobald keine völlige Hemmung mehr eintritt, neben anderen Kriterien die Abschwächung der Kontraktionen in Betracht zu ziehen und die Minderung des gesamten Arbeitsbetrags (oder der Energietransformation), — gemessen durch das Produkt aus der durchschnittlichen Verminderung der Schlagintensität und der Verminderung der Schlagzahl während der Dauer der Hemmungswirkung. Insbesondere stellte ich fest, daß, wenn möglich, mehr als ein Versuch gemacht werden muß, und man darauf sehen muß, die zu vergleichenden Werte wirklich in gleichem Maße auszudrücken, wenn brauchbare Vergleiche zwischen den bei verschiedenen Versuchen erhaltenen Resultaten gemacht werden sollen. Ich bemühte mich, »immer da, wo quantitative Resultate gegeben waren, die Vergleichsmethode genau anzugeben«.

2. Die Technik war eine außerordentlich verschiedene. Ich legte z. B. großen Wert auf eine getrennte Untersuchung der Hemmungswirkung bei Vorhof und Ventrikel und wählte eigens deshalb Gaskells Suspensionsmethode, weil sie weitaus das beste Mittel zum Vergleich von Veränderungen der Schlagkraft in beiden Kurven bot. Soviel ich weiß, hat keiner der anderen Forscher, welche den Temperatureinfluß auf den Hemmungsmechanismus des Froschherzens studiert haben, diese Methode benutzt. Eine Vergleichung meiner Kurven mit den seither veröffentlichten wird zeigen, wie sehr für diesen speziellen Zweck jede der anderen Methoden der von mir verwendeten nachsteht. Der Einwand Pretschistenskajas, daß bei der Suspensionsmethode

von Gaskell die Kontraktion unter unphysiologischen Umständen erfolge (womit, wie ich annehme, gemeint ist, daß die Kontraktion nicht dazu dient, den Flüssigkeitsdruck in dem Hohlorgan zu erhöhen), erscheint in bezug auf die in Betracht kommende Frage vollständig theoretisch, da die Hemmungswirkung des Vagus bei verschiedenen Herztemperaturen verglichen wird, ohne die mechanischen Bedingungen der Herzkontraktion zu ändern. Das gleiche gilt wohl auch für den von dem nämlichen Forscher erhobenen Einwand, daß bei Gaskells Methode das Herz nicht ernährt sei. Ich komme auf diesen Punkt später zurück. Ich kann aber nicht umhin, zu glauben, daß das von Pretschistenskaja erzielte Resultat nicht, oder wenigstens nicht in irgend erheblichem Grade davon herrührt, daß in seinen Versuchen das Herz ernährt wurde. (Kalbsblut, mit Salzlösung verdünnt.) Ich glaube, der Hauptgrund liegt darin, daß er sich auf das Studium der Änderungen in der Schlagfrequenz des Herzens beschränkte, ohne wenigstens in der durch Gaskells Methode ermöglichten präzisen Weise die vielleicht noch charakteristischeren Änderungen der Schlagkraft in Betracht zu ziehen und insbesondere ohne die Leistung des Vorhofs von der des Ventrikels zu trennen, was anscheinend bei der von ihm benutzten Suspensionsmethode nicht möglich war. Er prüfte die Erregbarkeit des Vagus durch den Minimalreiz, der eben deutlichen Stillstand hervorrief, oder häufig auch durch den schwächsten Reiz, der eine eben wahrnehmbare Verlangsamung des Herzschlags bei gewöhnlicher Temperatur bewirkte.

Er fand, daß diese Reizstärke identisch mit jener sei, die auch bei höherer oder tieferer Temperatur gerade Verlangsamung verursachte. Demgemäß schließt er daraus, daß die Erregbarkeit des Vagus von der Herztemperatur nicht beeinflußt wird. Ich selbst habe keine ähnlichen Versuche ausgeführt, bin vielmehr so verfahren, daß ich zuerst bei gewöhnlicher Temperatur einen deutlichen Hemmungseffekt durch Vagusreizung hervorrief und dann die Reizung bei höheren und tieferen Temperaturen wiederholte und das Gesamtergebnis verglich, wobei ich sowohl die Veränderungen der Schlagkraft wie auch die Veränderungen im

Rhythmus in Betracht zog. Die Reizintensität war meistens so gewählt, daß sie bei gewöhnlicher Temperatur keinen absoluten oder wenigstens keinen lang anhaltenden Stillstand verursachte, indem ich von der Annahme ausging, daß Veränderungen in der Wirkung bei höheren oder tieferen Temperaturen leichter festgestellt werden würden, wenn man von einer relativ geringen, jedoch vollkommen deutlichen Hemmungswirkung ausgehe. Meine Kurven geben einen sicheren Beweis dafür, daß ein Unterschied in der Hemmungswirkung viel leichter herausgebracht werden kann, wenn man eine Reizstärke anwendet, die bei der Anfangstemperatur ein Schwächerwerden der Schläge ohne völliges Aussetzen bewirkt, als wenn man für jede Temperatur jene Reizstärke sucht, bei der eben vollkommener Stillstand eintritt. Bei den tieferen Temperaturen treten die Veränderungen in der Schlagkraft (mit teilweiser Hemmung) weit deutlicher hervor als die Veränderungen im Rhythmus, und der Unterschied in der Hemmungswirkung, der sehr auffällig ist, wenn er durch die Veränderung der Herzkraft gemessen wird, kann sehr geringfügig sein und sogar übersehen werden, wenn man ihn bloß nach Veränderungen der Frequenz allein beurteilt.

Es ist vielleicht bemerkenswert, daß nicht ganz ein Jahr nach Pretschistenskajas Abhandlung Bassin[1]) eine Abhandlung veröffentlichte (ebenfalls aus dem Berner Laboratorium), in der er am Herzen der Landschildkröte nachweist, daß der Vagus ebenso leicht hemmend auf das Herz einwirkt, ob es nun erwärmt oder auf Zimmertemperatur gehalten sei. In der Tat zeigen, wie ich bereits an anderer Stelle erwähnte[2]), einige seiner Tabellen und Protokolle, daß die Tätigkeit der Hemmungsnerven bei den höheren Temperaturen sogar gesteigert wurde. (Beispiele: Versuch II u. VII.)

Die Betrachtung meiner Kurven zeigt, daß bei Aufzeichnung mit einem einfachen, am Herzen in situ befestigten Hebel, — wie bei Clark[3]), — es oft nicht möglich ist, eine vollkommen deutliche, allein oder vorwiegend auf die Kraft des Vorhofschlages beschränkte Hemmung zu entdecken, da die betr. Kurve notwendigerweise hauptsächlich oder ausschließlich Ventrikelkontraktionen wiedergibt.

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[1]) Arch. f. Physiol. 1907, Supp.-Bd. S. 429.

[2]) Amer. Journal of Physiology 1909, Bd. 24 S. 342.

[3]) Journal of Physiology 1912, Bd. 44 S. 169.

Ein viel besseres Urteil über die Zu- oder Abnahme der Hemmungswirkung läßt sich mit Hilfe von Gaskells Methode gewinnen. Man benutzt dann das Minimum und die Durchschnittshöhe der Kontraktionen während der Periode der partiellen Hemmung, die Länge der Periode, in der sich die Hemmungswirkung zeigt, und die Arbeit des Herzens pro Minute während und vor der Hemmung1) Als annäherndes Maß für die Arbeit kann die Ausdehnung der Kurve, multipliziert mit der Zahl der zugehörigen Herzschläge gelten.

3. Während die meisten Forscher bloß die Temperatur des Herzens allein zu ändern versuchten, die des Extrakardialmechanismus dagegen unverändert ließen, scheint doch nicht immer darauf geachtet worden zu sein, daß der Einfluß einer gegebenen Änderung in der Herztemperatur selbst auf die Tätigkeit der Hemmungsnerven nicht der gleiche zu sein braucht wie der Einfluß der nämlichen Temperaturänderung, wenn diese sowohl den ganzen Nervenmechanismus von der Medulla oblongata bis zum Sinus, als auch das Herz trifft. Clark2) z. B. tauchte Frösche, die »prone (?) auf einem Brett befestigt waren«, in eine Salzlösung, deren Temperatur rasch verändert werden konnte. Das Herz blieb in situ, der Kreislauf blieb anscheinend erhalten. Der Hemmungsmechanismus wurde durch in die Medulla oblongata eingeführte Elektroden ausgelöst. Offenbar wird hier, wenn die Temperatur der Lösung steigt, auch die Medulla oblongata samt den Zellen des Vaguskernes und den Nerven erwärmt, entweder unmittelbar durch die Lösung oder auf dem Wege des Blutes. Clarks Versuche können vielleicht zur

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1) Gesamthemmungswirkung $= n\left(1 - \frac{A'}{A}\right)$, wo $n$ die Dauer der Hemmung in Minuten entspricht; $A'$ das Produkt aus der Schlagzahl (pro Minute) während der Hemmung und der durchschnittlichen Größe der Schläge, und $A$ den entsprechenden Wert vor der Hemmung darstellt.

2) loc. cit.

Beantwortung der Frage dienen, inwiefern eine Steigerung der Temperatur des ganzen nervösen Hemmungsmechanismus mit Einschluß des Herzens die Reaktion auf eine gleichzeitige, direkte Reizung des Vaguskernes und seiner Wurzelfasern beeinflußt; sie sind aber sicherlich verschieden von den Versuchen Luchsingers und Ludwigs, Pretschistenskajas und von meinen, in denen die Temperaturveränderung soweit als möglich auf das Herz beschränkt blieb. Es ist daher nicht verwunderlich, daß seine Resultate von denen der erwähnten Forscher abwichen. Während Pretschistenskaja findet, daß die Erregbarkeit des Vagus innerhalb eines weiten Bereichs von Herztemperaturen unverändert bleibt, — Ludwig und Luchsinger, wie ich selbst, dagegen der Meinung sind, daß sie bei Temperatursteigerung sogar zunimmt, — kommt Clark zu dem Ergebnis, daß eine Steigerung der Temperatur über Zimmertemperatur eine bedeutende Verminderung der Erregbarkeit des Hemmungsmechanismus hervorrufe.

Es ist ganz gut möglich, daß dieselbe Temperatur, welche die Erregbarkeit des Hemmungsmechanismus im Niveau des Vaguskernes aufhebt, bei Beschränkung auf das Herz, die hemmende Wirkung der Vagusfasern erhält oder sogar steigert.

Auch Clarks zweite Versuchsreihe hat wenig Berührungspunkte mit meinen Versuchen. »Die Temperatur wurde an verschiedenen Punkten der Herzoberfläche erhöht durch Berührung mit der Spitze eines Heizkonus nach v. Frey. Die berührte Fläche hatte stets nur einen Durchmesser von 1 mm.« Er fand, daß lokale Erwärmung an der Sinus-Vorhofgrenze Verlust oder Abnahme der Hemmungswirkung verursachte. Ebenso trat keine, oder verminderte Hemmung ein, wenn der an den rechten Vorhof angrenzende Sinusteil erwärmt wurde. Erwärmung anderer Teile des Sinus oder des übrigen Herzens war dagegen ohne Einfluß auf die hemmende Wirkung des verlängerten Markes. Man kann das Ergebnis wohl auffassen als eine Störung in der Leitfähigkeit der Ganglienzellen oder deren Synapsen, die in die Bahn der Hemmungsnerven eingeschaltet sind (vorübergehende Lähmung durch Hitze).

Wenn man den Einfluß der Herztemperatur auf die Wirksamkeit des Vagus prüfen will, ohne die Nerventemperatur zu ändern, muß man solche Temperaturen und Erwärmungsmethoden benutzen, daß die Erregungsleitung distal von dem Reizorte nicht oder so wenig wie möglich beeinträchtigt wird.

4. Von den verschiedenen Forschern sind Herzen verschiedener Tiere verwendet worden. Es sollte beachtet werden, daß größte Vorsicht selbst dann am Platze ist, wenn Versuche an Herzen verschiedener Kaltblüter verglichen werden. Es ist klar, daß der Vergleich zwischen kalt- und warmblütigen Herzen noch schwieriger ist. Selbst bei Fröschen derselben Art dürfte, wie Clark sehr richtig bemerkt, die Länge der Zeit, innerhalb der sie im Laboratorium gehalten wurden, ein nicht zu übersehender Faktor sein.

Wenn man die Vaguswirkung auf das Herz prüft bei einer Temperatur, welche der lähmenden nahekommt, so muß man bedenken, daß die Tätigkeit des Nervus accelerans ebenfalls und in höherem Maße noch als die der Hemmungsfasern durch Wärme gesteigert wird. Daher kommt es, daß bei den höheren Temperaturen die Hemmungsfasern zwar ganz gut wirken, aber die Wirkung ist manchmal vorübergehend und die Hemmung kann von einer deutlichen Beschleunigung gefolgt sein. Besteht die Hemmung vorwiegend in einer Verminderung der Schlagkraft (die infolge der hohen Temperatur überhaupt schon gering ist) und betrifft sie gar nur den Vorhofschlag, so kann sie sich dem Nachweis entziehen, wenn man nicht Gaskells Registriermethode benutzt.

Die von mir mitgeteilte Beobachtung, daß die Tätigkeit der Beschleunigungsfasern regelmäßig bei Steigerung der Herztemperatur zu-, bei Temperaturerniedrigung abnimmt, ist von allen folgenden Forschern[1]) bestätigt worden. Dies zeigt, daß sie leichter zu beobachten ist als das entsprechende Phänomen bei den Hemmungsfasern, eine Bemerkung, die ich in meiner ersten Abhandlung gemacht habe. Der Grund dafür, oder wenigstens ein wichtiger Grund liegt, wie ich annehme, im Fehlen irgendeines besonders empfindlichen Verbindungsgliedes im Verlauf der Beschleunigungsfasernbahn, die plötzlich leitungsunfähig wird, sobald die kritische Temperatur erreicht ist. Das Vorhandensein einer solchen Verbindung auf der Hemmungsbahn gibt die natürlichste Erklärung für die von mehr als einem Forscher, einschließlich Clark für den Frosch und E. C. Martin[1]) für die Sumpfschildkröte aufgestellte Behauptung, daß manchmal bei einer Temperatur, die an sich nicht hoch genug war, den Herzschlag zu hemmen, durch Reizung des Vagus mit Strömen irgendwelcher Stärke dennoch keine Wirkung hervorgebracht wurde, obgleich nach Abkühlung wieder Hemmung erzielt werden konnte. Dies zeigt die zeitweise völlige Funktionsunfähigkeit eines Teiles des Leitungsweges an, eine Funktionsunfähigkeit, die in meinen Versuchen vermieden worden zu sein scheint. Wie bereits erwähnt, legte ich großen Wert auf die Vorhofkurven, die in der Regel für Temperaturen benutzt werden konnten, bei denen sich der Ventrikel in Hitzelähmung befand. Es ist natürlich wohl bekannt, daß Sinus und Vorhof beträchtlich höhere Temperaturen vertragen können als der Ventrikel.

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[1]) Cf. Hatschek, Arch. f. d. ges. Physiol. 1905, Bd. 109 S. 199; Frank, Zeitschr. f. Biol. 1907, Bd. 49 S. 392.

Was die in meiner früheren Abhandlung aufgestellten Schlußfolgerungen betrifft, so kann ich nur sagen, daß ich nach sorgfältiger Wiederprüfung meiner sehr zahlreichen Kurven nicht imstande bin, sie irgendwie anders auszulegen. Meine sämtlichen Folgerungen fußen auf diesen Kurven. Aufzeichnungen, die unter anderen Bedingungen oder mittels weniger geeigneter Methoden gewonnen sind, können anscheinend zu abweichenden Ergebnissen führen. Ich kann nur sagen, daß meine Kurven mit Hilfe der verwendeten Versuchsmethoden und unter den durch sie geschaffenen Bedingungen wirklich von den Batrachierherzen geschrieben worden sind. Sie müssen daher irgendeine Bedeutung haben, und die Frage ist nur, ob ich sie richtig erklärt habe. Kein späterer Forscher hat irgendeine andere Erklärung dafür vorgeschlagen, und ich glaube auch nicht, daß jemand, der auch nur die beschränkte Anzahl meiner in der früheren Abhandlung veröffentlichten Kurven sorgfältig prüft, irgendeine andere Schluß-

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1) Amer. Journ. of Physiology 1904, Bd. 11 S. 387.

folgerung als die meine daraus ziehen kann. Kann z. B. jemand daran zweifeln, daß in Fig. 1—6 der früheren Abhandlung die Gesamthemmungswirkung bei tieferen Temperaturen geringer und bei höheren größer ist als bei mittleren Temperaturen? Ich will nicht näher darauf eingehen, ob man die hier besprochene Wirkung als eine Änderung in der Erregbarkeit des Vagus auffassen soll. In meiner Abhandlung habe ich mich absichtlich enthalten, von der Erregbarkeit des Vagus oder des Sympathicus zu sprechen, und sprach statt dessen von der »Wirkung« oder höchstens von der Wirksamkeit des Nerven, die gesteigert oder vermindert wird. Man müßte, wie ich glaube, überkritisch sein, wollte man leugnen, daß in den erwähnten Figuren die Änderung in der Vaguswirkung trotz konstanten Reizes mit dem Wechsel der Herztemperatur zusammenhängt. Die Reizung verursacht beinahe vollständiges Aussetzen des Vorhof- und Ventrikelschlages — für das Herz bei gewöhnlicher Temperatur (15,5°) —, während bei 8° beide Herzteile ganz deutliche, jedoch sehr verminderte Schläge zeichnen. Bei noch tieferer Temperatur (Fig. 3 u. 4) ist die Abnahme der Kontraktionsgröße von Vorhof und Ventrikel während der Vagusreizung noch geringer, wenn auch völlig deutlich.

In Fig. 5, bei viel stärkerem Reiz, — Herz liegt noch in der kalten Lösung — ist die Gesamthemmungswirkung deutlich geringer als in Fig. 1. In Fig. 6 — Temperatur 13° — ist die Hemmung bei ursprünglicher Reizstärke beinahe, aber nicht ganz so groß wie in Fig. 1 — bei Temperatur 15,5° — und zeigt dadurch, daß die Resultate bei tieferer Temperatur nicht etwa von einer dauernden Änderung in der Vaguswirkung herrühren. Es wurde natürlich, wie die erwähnten Figuren beweisen, die selbstverständliche Vorsichtsmaßregel nicht außer acht gelassen, am Schlusse einer Serie von Beobachtungen bei höherer und tieferer Temperatur die Vaguswirkung auf das Herz bei oder nahe der ursprünglichen Mitteltemperatur zu prüfen. Pretschistenskajas Behauptung: »Diese Maßregel ist sehr wichtig, aber ich vermisse sie in den oben zitierten Arbeiten«, rührt zweifellos von einem Versehen her. Luchsingers und Ludwigs Protokolle zeigen deutlich, daß auch sie diese Maßregel nicht vernachlässigten. Seine Behauptung, meine Untersuchung »ist unter Leitung von Gaskell ausgeführt worden«, muß ebenfalls richtiggestellt werden, um Dr. Gaskell gerecht zu werden, der in keiner Weise, weder für die Inangriffnahme noch für Durchführung dieser Versuche direkt verantwortlich war, obgleich er mir im Lauf der Arbeit über verschiedene Punkte gütigst Rat erteilte. Indirekt verantwortlich, im besten Sinn des Wortes, war er natürlich insofern, als ich niemals den Impuls gefühlt hätte, einen wenn auch bescheidenen Beitrag zur Kenntnis des Mechanismus der Herzregulation zu liefern, wäre es nicht aus Begeisterung für seine Arbeit über die Physiologie des Herzens geschehen.

Da nur eine beschränkte Anzahl der in der früheren Abhandlung veröffentlichten Kurven die hier in Betracht kommende Frage behandelt, welche an sich selbst wiederum nur einen Abschnitt meiner Untersuchung bildete, so werden hier weitere Kurven folgen[1]).

Sie erläutern drei Punkte: 1. die Wirksamkeit der Hemmungsfasern an der Grenze der Wärmelähmung des nach Gaskells Methode suspendierten Herzens;

2. die Steigerung der Hemmungswirkung bei Temperaturerhöhung des suspendierten Herzens und Verminderung bei Temperaturabnahme — gleichbleibende Reizstärke vorausgesetzt;

3. Bestätigung von 1 und 2 am Herzen in situ.

## I.

## Es kann vollständige Hemmung eintreten wenn das Herz nahe der Wärmelähmung ist.

Wegen Raummangels war es nicht möglich, in der früheren Abhandlung eine genügende Kurvenanzahl abzubilden, um diese

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[1]) Die Zeitmarken bedeuten in allen Figuren Intervalle von 2 Sekunden und nur in Fig. 12 Sekunden. Vagusreizung wird durch »V« angezeigt. Steht eine Zahl dabei, z. B. V 50, so bedeutet dies die Entfernung der Induktionsrollen in Millimetern. Es wird immer das gleiche Induktorium benutzt, mit einem Daniellelement (immer dasselbe) im primären Kreis. Die Richtung, in der die Kurven abzulesen sind, ist durch Pfeile angezeigt.

Behauptung näher zu begründen. Es ist jedoch nichts leichter zu beweisen, wie Fig. 1 u. 2 dieser Abhandlung zeigen.

Fig. 1 und 2 stellen Kurvenabschnitte dar, die von einem Krötenherz registriert wurden. Ehe das Herz in der Klammer befestigt wurde, verursachte Reizung des Vagus Hemmung; nach der Suspension des Herzens aber wurden bei Lufttemperatur mit jeder Reizstärke nur noch reine Beschleunigungen erzielt.

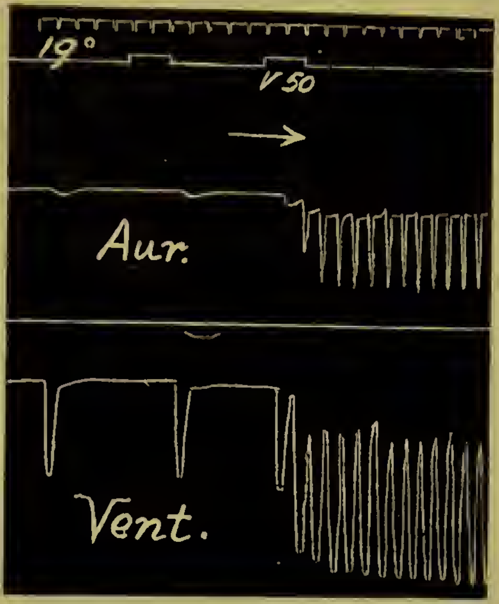

Fig. 1.
Vorhof- und Ventrikelkurven (Krötenherz); V 50 = Reizung des Vagus (50 mm Rollenabstand.) — Herz bei Lufttemperatur (19°).

Ein Befeuchten des Nerven stellte die Hemmungswirkung nicht wieder her. Fig. 1 zeigt die Wirkung einer Vagusreizung bei 50 mm Rollenabstand und 19° C. Hierauf wurde Salzlösung von 34,5° eingeleitet (24 Sekunden vor Beginn der Fig. 2). Nach einiger Zeit hörte der Ventrikel zu schlagen auf, während der Vorhof rasch, aber schwach weiterschlug. V 50 bewirkte völligen Stillstand des Vorhofs, von gesteigerter Tätigkeit gefolgt, währenddessen der Ventrikel eine Reihe kräftiger Schläge machte. Sie begannen erst einige Zeit, nachdem der Vorhof wieder zu schlagen angefangen hatte und setzten bald wieder aus. Der Vorhof schlug schwach weiter. Vagusreizung mit gleichem Rollenabstand (50 mm) verursachte vollständigen Stillstand des Vorhofs, von einem Ausbruch von Schlägen gefolgt, in die dann der Ventrikel einfiel. Mit dem Vergrößerungsglas kann man sehen, daß der Vorhof zweifellos bis zu dem Augenblick geschlagen hatte, wo Vagusreizung erfolgte.

Am Schluß des in Fig. 2 abgebildeten Kurvenabschnittes wurde die Flüssigkeit abgeleitet. Das Herz hörte zu schlagen auf. Vagusreizung mit gleichem Rollenabstand (50 mm) bewirkte eine Reihenfolge kleiner Schläge, an denen sowohl Vorhof als Ventrikel bei Lufttemperatur teilnahmen. Nun wurde eine Lösung von 34° C eingeleitet, und es trat Stillstand ein. V 50 brachte den Vorhof zu kräftigem Schlagen, und etwas später fiel der Ventrikel plötzlich mit einer Reihenfolge enormer Schläge ein (nicht abgebildet). Es ist nur die Wirkung der Beschleunigungsfasern, die an der Kurve gezeigt werden konnte, da völliger Stillstand eingetreten war, noch ehe man den Vagus reizte. Es ist jedoch ganz gut möglich, daß, wie schon in der früheren Abhandlung bemerkt, die Hemmungsfasern auch während des Stillstandes eine Wirkung hervorgebracht haben.

Bei einigen dieser Kurven scheint ein solcher Hemmungseffekt durch eine leichte Abnahme im Tonus angedeutet zu sein. Durch Reizung des Vagus konnte nun eine Hemmung des auf Zimmertemperatur abgekühlten Herzens

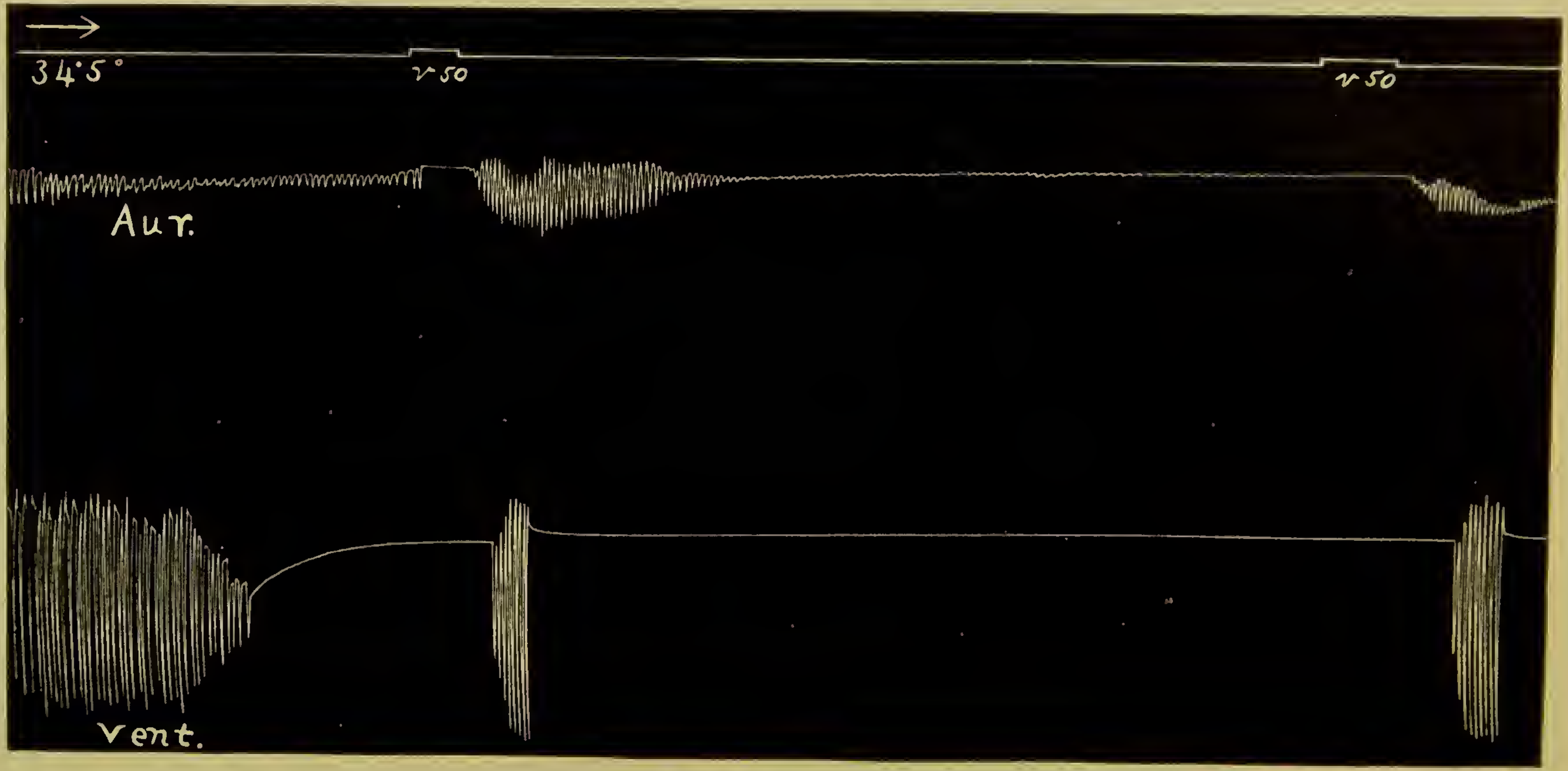

Fig. 2.

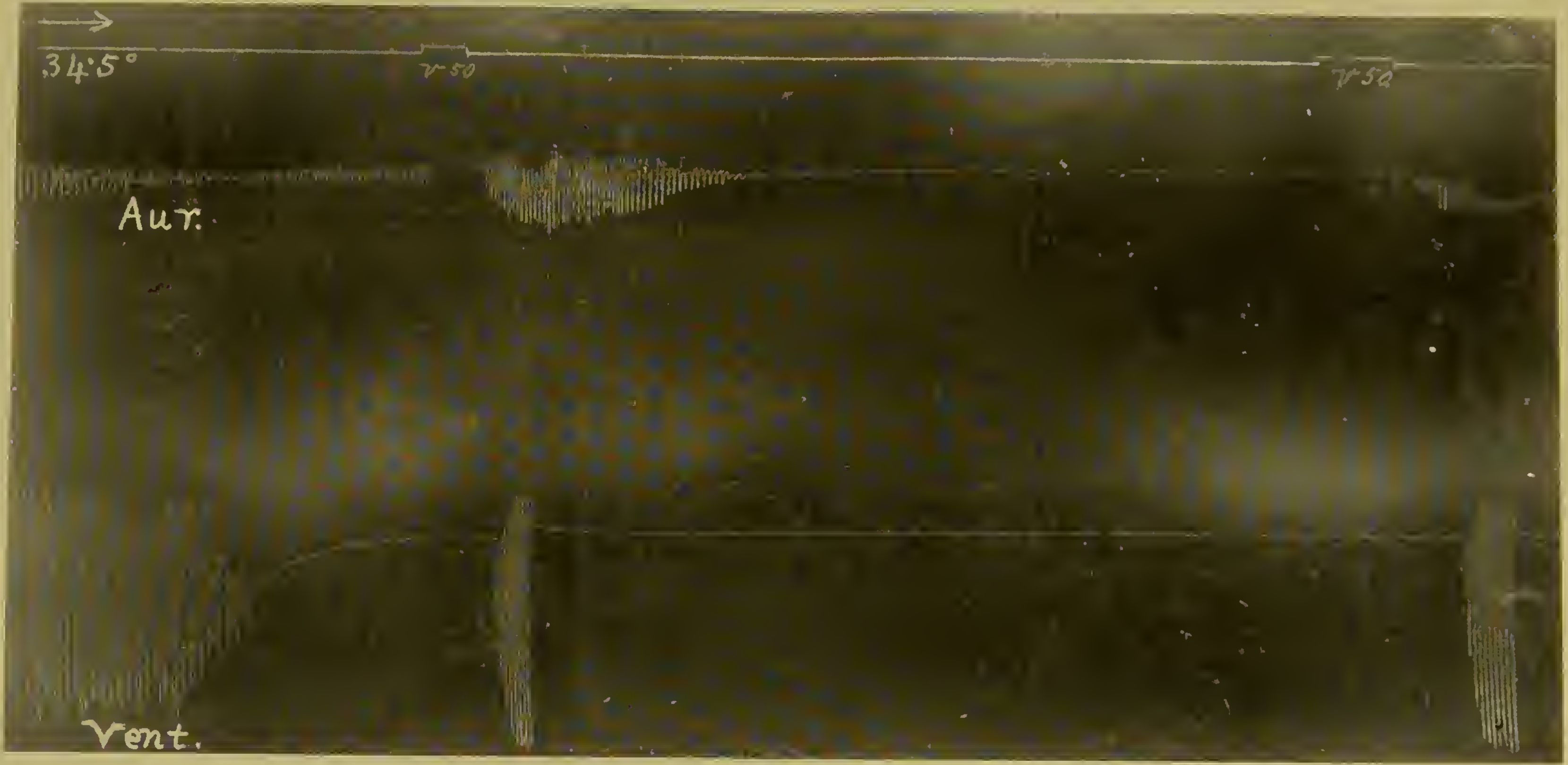

Fig. 2.

Gleiches Herz wie in Fig. 1. — V 50: Vagusreizung; (Herz in Lösung von 34,5° C;) zuerst während Vorhof noch sehr gut schlägt und dann infolge Hitzelähmung nahe dem Stillstand sich findet. Sowohl Vorhof- wie Ventrikelkurve wiedergegeben. Die Trommel hatte die gleiche Umdrehungszahl wie in Fig. 1 — aber Zeitangabe ist unvollkommen.

bewirkt werden. Der Nerv war während des Versuchs weder benetzt noch eingetaucht worden.

In Fig. 3 ist nur die Vorhofkurve abgebildet (Froschherz).

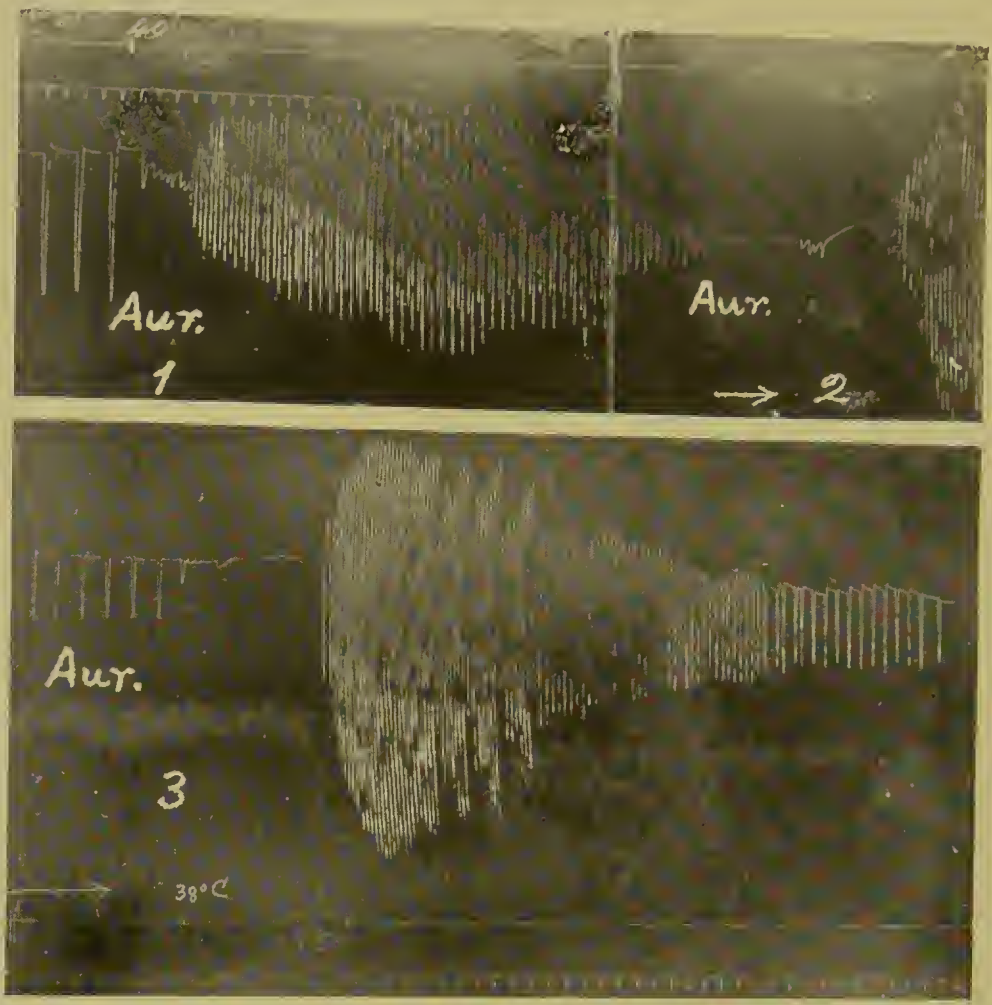

Fig. 3.

Froschherz; nur Vorhofkurve wiedergegeben. In Nr. 1 (bei 40°) wurde eine Lösung von 40° eingeleitet. Zwischen 1 und 2 liegt ein Zeitraum von etwas über 1 Minute, während dessen das Herz in der 40° Lösung weiterschlug. — In Nr. 2 trat durch Reizung des Vagus bei V 50, als der Vorhof schon nahe daran war, seine Tätigkeit einzustellen, ein vollständiger Stillstand ein, gefolgt von einer bedeutenden Beschleunigung und Verstärkung. Bei Nr. 3 hatte das Herz bei Lufttemperatur geschlagen, bis am angegebenen Punkt eine Lösung von 38° eingeleitet wurde. Vorhof ging in Stillstand über. Reizung des Vagus (V 50) verursachte keinen ersichtlichen Hemmungseffekt, ausgenommen vielleicht ein leichtes Absinken des Tonus (Steigen des Hebels). Aber die Beschleunigungswirkung ist sehr deutlich gekennzeichnet.

In Nr. 1 dieser Figur wurde bei dem mit »40°« bezeichneten Punkt

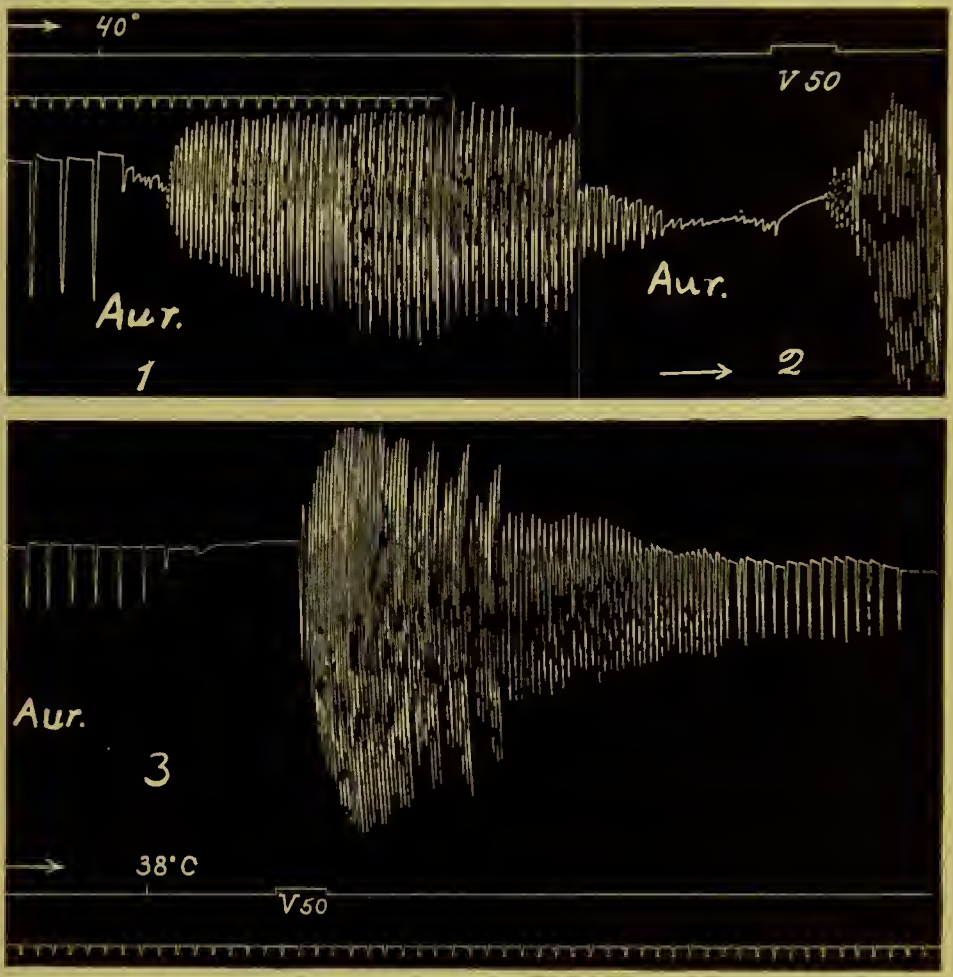

Fig. 3.

eine Salzlösung von 40° eingeleitet. Einige Forscher, einschließlich Pretschistenskaja, haben eine Hemmung beschrieben, die durch plötzliche Steigerung der gewöhnlichen Herztemperatur verursacht wurde. Die Kurve läßt klar erkennen, daß das, was sich in diesem Versuch ereignete, aus einer plötzlichen Steigerung der Schlagfrequenz bestand, die aber anfangs von einer großen Kraftverminderung begleitet war. Diese Verminderung dauerte einige Sekunden lang, und dann nahmen die Schläge rasch wieder an Intensität zu, während sie ihre Frequenz beibehielten oder sogar beschleunigten. Das Bild ist keineswegs das einer Hemmung, sondern macht eher den Eindruck, als ob als erste Wirkung der gesteigerten Herztemperatur der Rhythmus der automatischen Entladung beschleunigt werden sollte und das Vorhofgewebe noch nicht imstande wäre, schnell genug die nötige Energie auszulösen, um auf jeden Impuls zu reagieren, es sei denn durch Schläge, weit schwächer als die, wie sie bei gewöhnlicher Temperatur ausgeführt würden. Die während der ersten paar Sekunden geleistete Gesamtarbeit wird durchaus nicht vermehrt, wenn nicht sogar eher verringert.

Beobachtung 2 ist eine Fortsetzung der Kurve — Zeitabstand zwischen 1 und 2 = 1 Minute. Die Ventrikelkontraktion hatte aufgehört, der Vorhof schlug schwach weiter. Auf Vagusreizung trat völliger Stillstand ein mit deutlicher Abnahme des Tonus, gefolgt von ausgeprägter Beschleunigungswirkung.

Bei Nr. 3 der gleichen Figur schlug der Vorhof wieder bei Lufttemperatur, bis eine Lösung von 38° C eingeleitet wurde. Es erfolgt beinahe augenblickliches Aufhören der Schläge, was aber, wie viele Versuche beweisen, nicht von einer Hemmung herrührt, sondern Wärmestillstand anzeigt. Das unterscheidende Merkmal besteht darin, daß sich das Herz von einer bloßen Hemmung erholt, wenn man es sich selbst überläßt, nicht aber von einem Wärmestillstand, es sei denn, daß man die Temperatur erniedrigt. Reizung des Vagus mit gleicher Reizstärke während des Stillstandes führte zu einer Reihe von Schlägen.

Hätte man sich auf eine Kurve beschränkt, welche die Tätigkeit des Ventrikels allein oder vorwiegend zum Ausdruck bringt, wie es bei den Kurven jener Forscher der Fall ist, die über abweichende Resultate berichten, so wäre, wie leicht einzusehen, es schwer gewesen, nach dem Aufhören des Ventrikelschlages die Hemmungswirkung noch zu erkennen (in Fig. 3 Nr. 2), die doch bei Gaskells Methode so unzweideutig hervortritt.

In der Tat würde man wohl aus Nr. 2 u. 3 dieser Figur die gleiche Folgerung gezogen haben, daß die Hemmungsfasern des Vagus bei dieser hohen Herztemperatur unwirksam wären.

In Fig. 4 und 5 (Kurven eines Krötenherzens, das vorher 110 Sekunden lang in eine Salzlösung von 35° C und dann 85 Sekunden lang in eine solche von 38,5° C getaucht worden war) war der Ventrikel zu völligem Stillstand gelangt, während der Vorhof noch schwach weiterschlug, wie man durch Betrachtung der Kurve mit einem Vergrößerungsglas sehen kann (Fig. 4 Nr. 1). — Vagusreizung mit 70 mm Rollenabstand verursachte während eines 8—9 Schlägen entsprechenden Zeitraumes vollständiges Aussetzen des Vorhofschlages, gefolgt von einem Ausbruch von Kontraktionen, in die der Ventrikel einfiel. Das Herz wurde dann in eine Lösung von 18,5° C (Fig. 4 Nr. 2) getaucht und der Vagus mit dem bisherigen Rollenabstand gereizt.

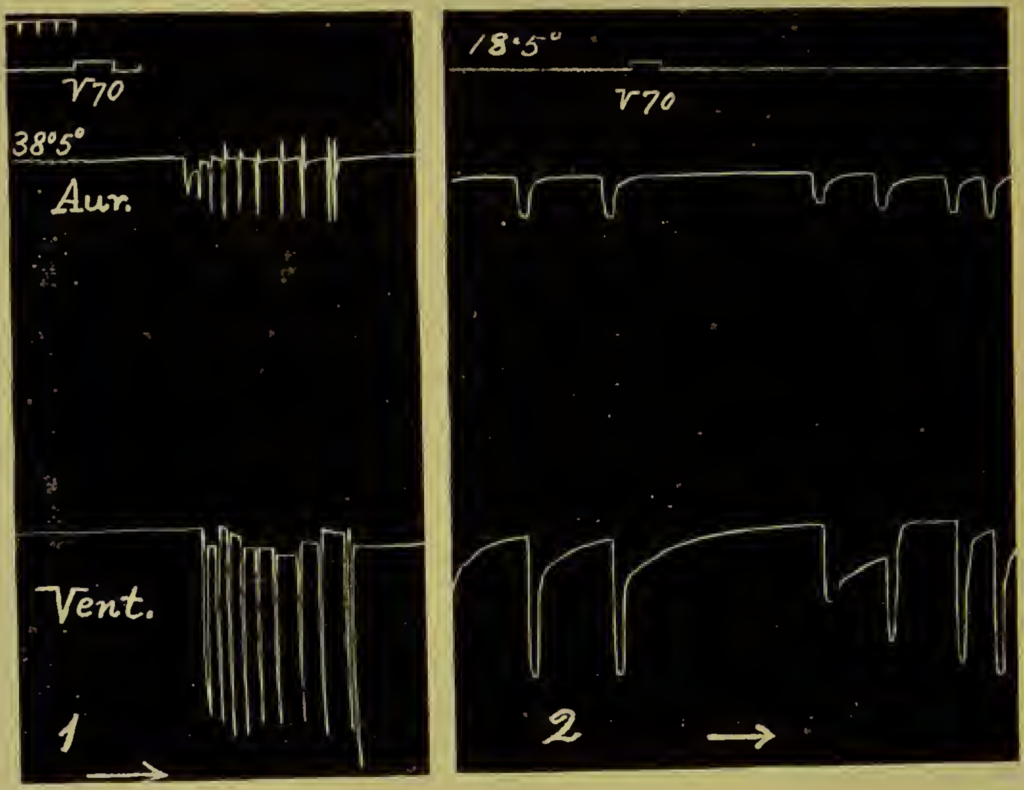

Fig. 4.

Krötenherz. Bei Nr. 1, nach 110 Sekunden langem Eintauchen des Herzens in Lösung von 35° und dann 85 Sekunden lang in 38,5°, hatte Ventrikel zu schlagen aufgehört, und Vorhof befand sich an der Grenze beginnender Hitzelähmung. (Kurve ist mit Vergrößerungsglas zu untersuchen.) Vagusreizung mit V 70 verursacht völliges Aufhören der Schläge, von einer zweiten Steigerung des Vorhofschlages gefolgt, während die Ventrikelschläge wieder einsetzen. In Nr. 2 — Herz bei 18,5° — wurde durch V 70 ebenfalls Hemmung erzielt.

Nun trat ein der Dauer von 2 oder 3 Schlägen entsprechender Stillstand ein, auf welchen wieder gesteigerte Tätigkeit folgte. Als eine Lösung von 34,3° C (Fig. 5) an Stelle der kälteren trat, gingen Vorhof und Ventrikel fast augenblicklich in Stillstand über. V 70 blieb ohne Wirkung; dagegen erzielte V 40 einen Ausbruch sowohl von Vorhof — wie auch von Ventrikelschlägen. Zwischen Fig. 5 und 6 verstrich ein Zeitraum von 20 Sekunden. In dieser Zwischenzeit verfiel das Herz wieder in Stillstand, wurde aber durch Vagusreizung mit 60 mm Rollenabstand aufs neue zum Schlagen gebracht (Fig. 6).

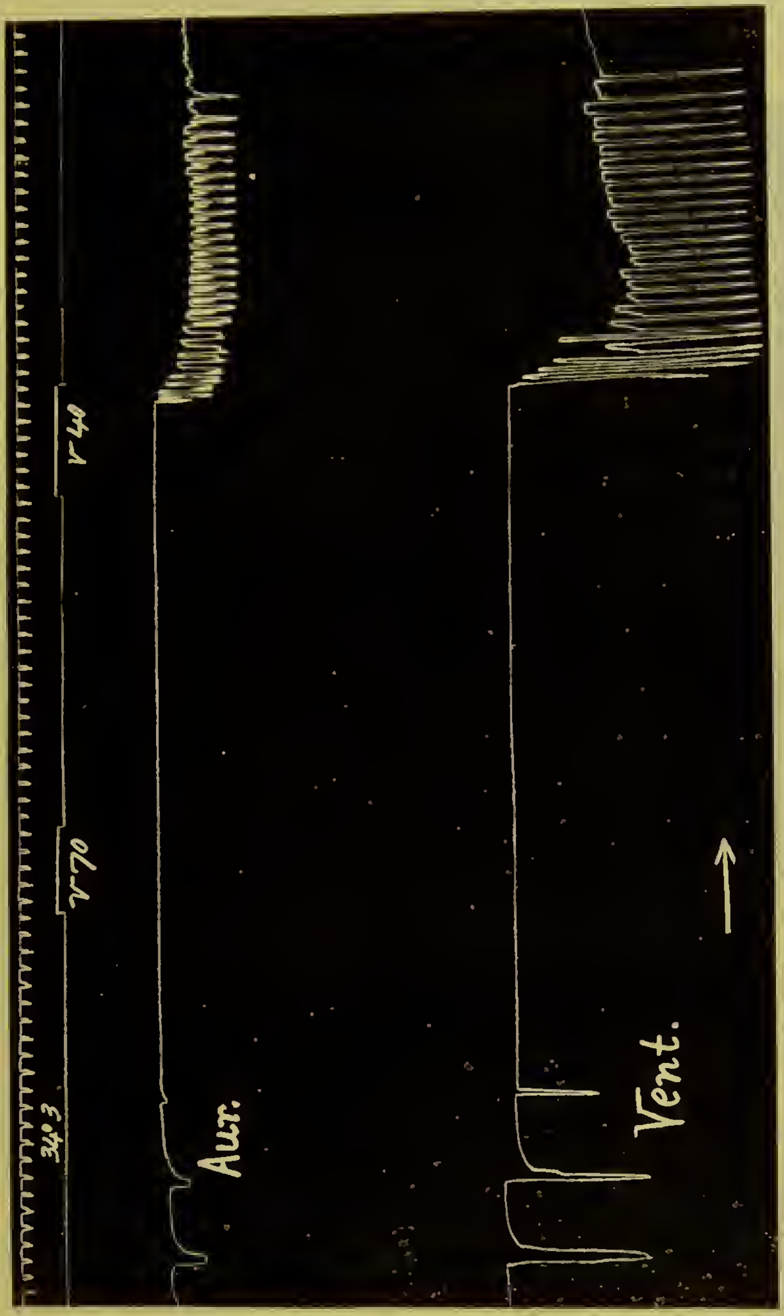

Fig. 5.

Dasselbe Herz wie in Fig. 4, aber in etwas späterer Periode des Versuches. In der Lösung von 34,3° hört das Herz plötzlich zu schlagen auf. V 70 bleibt ohne Wirkung, aber eine stärkere Vagusreizung (V 40) verursacht, sowohl bei Vorhof als bei Ventrikel, Wiedereinsetzen der Schläge.

Mit gewöhnlichen Methoden wäre es unmöglich gewesen, in Nr. 1 Fig. 4 die Hemmung zu erkennen, die jedoch in der Vorhofkurve unter dem Vergrößerungsglas außerordentlich deutlich zutage tritt. Die Hemmung ist sogar eine größere als in Nr. 2 Fig. 4 bei 18,5° C, wenn man sie nach der Anzahl der unterdrückten Schläge berechnet. Die Tatsache, daß in Fig. 5 (bei V 70) eine Reizung des Vagus ohne Wirkung blieb, kommt für die Wirksamkeit der Hemmungsfasern nicht in Betracht. Denn das Herz war zum Stillstand gekommen und die fragliche Reizstärke genügte nicht, um bei diesem Herzzustand Beschleunigung zu verursachen. Es waren sicher nicht alle Fasern des Nerven gelähmt, da das Herz auf eine stärkere Reizung des Vagus (40 mm Rollenabstand) ja sogar auf eine solche mit 60 mm Rollenabstand ganz gut reagierte, und zwar auf dem einzig möglichen Weg der Beschleunigung (Fig. 6).

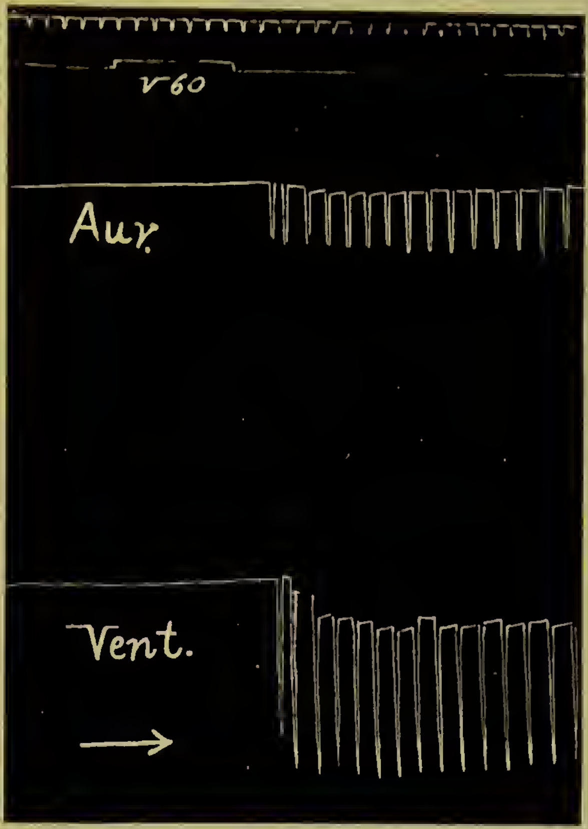

Fig. 6.
Gleiches Herz wie bei Fig. 4 u. 5, aber später gezeichnet. Gleiche Temperatur wie in Fig. 5. V 60 verursacht eine Gruppe von Schlägen, die an Frequenz allerdings der in Fig. 5 durch V 40 erzielten nachstehen.

Auf Grund dieser und ähnlicher Kurven kann ich nur meine frühere Angabe bestätigen, daß die Hemmungswirkung des Vagus bis an die Grenze der Wärmelähmung des Frosch- (und Kröten)herzens nachweisbar bleibt.

Es sollte vielleicht nochmals betont werden, daß bei unseren Versuchen zur Erzeugung der Wärmelähmung die Temperatur in der Regel so niedrig als möglich gehalten wurde. Zu dem

Zwecke wurde das Präparat zunächst einige Zeit bei einer Temperatur belassen, die nicht hoch genug war, um unmittelbar Stillstand zu erzeugen. Auf diese Weise gelingt es möglicherweise das Versagen der in die Hemmungsbahn eingeschalteten Ganglienzellen zu vermeiden.

## II.

Was den Einfluß einer Steigerung der Herztemperatur auf die Tätigkeit der Hemmungsfasern im allgemeinen betrifft, so ist darauf schon bei einigen Figuren der früheren Abhandlung Bezug genommen worden. Da indessen der einschlägige Abschnitt jener Abhandlung nur wenige Beispiele enthält, die sich außerdem zumeist auf Temperaturen unter der Norm bezogen, so werden hier einige weitere Kurven folgen (Fig. 7—10), welche sich hauptsächlich auf Temperaturen über Lufttemperatur beziehen.

In Fig. 7—10 sind Vorhofkurven eines Froschherzens wiedergegeben. Der Vagus wurde immer mit gleichem Rollenabstand gereizt.

Fig. 7 Nr. 1 zeigt die Wirkung einer Vagusreizung von 12 Sekunden Dauer bei Lufttemperatur. Der Vorhofschlag ist nicht völlig gehemmt. Während ungefähr 35 Sekunden ist eine Hemmungswirkung sichtbar, die 6—7 Schlägen der ursprünglichen Frequenz entspricht. Bei dem in Nr. 2 angegebenen Punkt wurde Salzlösung von 30° C eingeleitet. Eine Reizung des Vagus während ungefähr 11 Sekunden (Nr. 3) — Herztemperatur andauernd 30° — verursachte völligen Stillstand des Vorhofs, und während mindestens 35 Sekunden machte sich eine Hemmung bemerkbar, die ungefähr 18 Schlägen der Anfangsfrequenz, vor Erregung des Vagus, entsprach.

In Fig. 8 wurde die Lösung durch eine solche von 38° C ersetzt. Reizung des Vagus auf die Dauer von nur 8 Sekunden (eine entschieden kürzere Zeit als in Fig. 7) verursachte vollständiges Aussetzen des Vorhofschlages mit Verminderung im Tonus. Während mindestens 32 Sekunden kann man eine Hemmung beobachten, die auf jeden Fall 13 Schlägen entspricht. — Wiederholung der Vagusreizung an dem Herzen bei 38° ergab eine anscheinend noch längere Dauer der Hemmungswirkung. Es muß indessen bemerkt werden, daß bei diesen außergewöhnlichen Temperaturen nicht immer zu entscheiden ist, ob eine Verminderung in der Stärke der Kontraktionen schon die kommende Lähmung ankündigt oder als Hemmung noch aufgefaßt werden darf.

Auf jeden Fall ist es vollkommen klar, daß selbst bei der relativ hohen Temperatur von 38° der Hemmungsmechanismus völlig aktiv ist, sogar in höherem Maße als bei 13° C, wenn das vollständige Aufhören der Schläge oder die Anzahl der Schläge, über welche sich die Hemmungswirkung erstreckt, als Kennzeichen genommen wird.

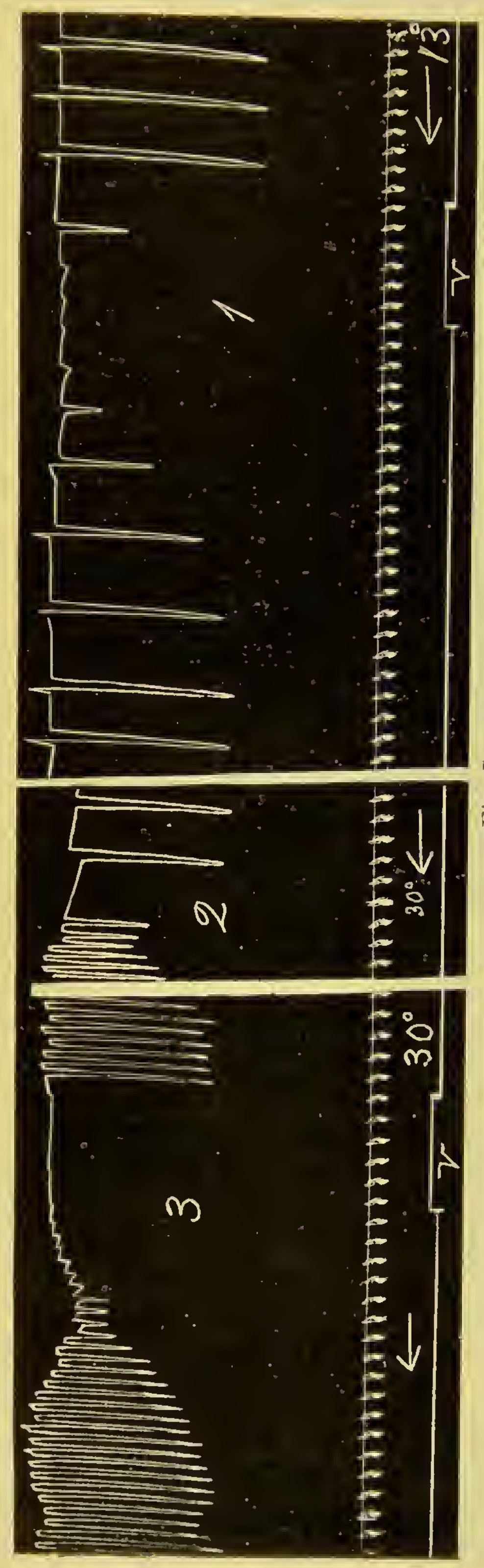

Fig. 7.

Froschherz. Nur Vorhofkurve abgebildet. Gleiche Reizstärke wurde für den Vagus verwendet. — Herz bei Lufttemperatur (13⁰) in Nr. 1 und 30⁰ in Nr. 3. — Hemmungswirkung ist bei 30⁰ größer. Beim angegebenen Punkt wurde die Lösung von 30⁰ eingeleitet (in Nr. 2). Zwischen 2 u. 3 verging ein Zeitraum von ungefähr 30 Sekunden.

In Fig. 9 schlug das Herz wieder am Anfang der Kurve bei 13⁰ C. Die Vorhofschläge waren viel rascher als bei Beginn des Versuchs (Fig. 7 Nr. 1) und ihre Kraft dementsprechend geringer. Der Hemmungseffekt der Vagusreizung bei gleicher Reizstärke war größer als in Fig. 7[1]).

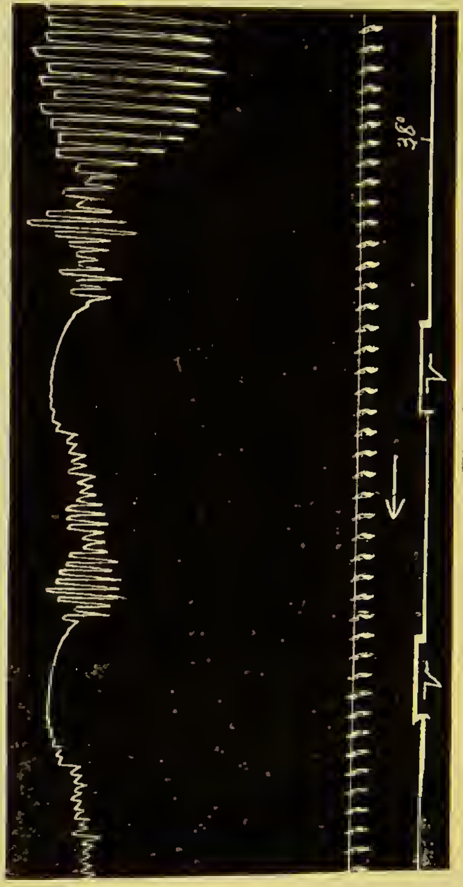

Fig. 8.

Gleiches Herz wie in Fig. 7. Nur Vorhofkurve wiedergegeben. Gleiche Reizstärke. Herz schlug in der Lösung von 30⁰ weiter, bis sie am bezeichneten Punkt mit einer Lösung von 38⁰ vertauscht wurde. Vagus zweimal gereizt, Hemmung in beiden Fällen ganz deutlich.

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[1]) Es ist wohl möglich, daß es nicht die Temperatur des Herzens als solche ist, welche die Größe der Hemmungswirkung des Vagus bestimmt, sondern der Wechsel in der Schlagfrequenz (bzw. der sie be-

Als die bisherige Lösung durch eine solche von 44° C ersetzt wurde, erfolgte augenblicklich vollständiger Stillstand. Reizung des Vagus verursachte deutliche Zunahme im Tonus (vom Nervus accelerans herrührend), worauf der Vorhofschlag wieder einsetzte. Es könnte nun leicht voreilig angenommen werden, daß der Hemmungsmechanismus bei dieser Temperatur inaktiv sei. Bei der nächsten Vagusreizung zeigt sich jedoch, daß diese Annahme irrig wäre. Die Vorhofschläge wurden pünktlich gehemmt, und der Schreibhebel verzeichnete eine Horizontallinie. Die nachträgliche Wiederaufnahme der Herztätigkeit wurde durch eine Steigerung im Tonus angekündigt, und dann erst setzten die Schläge ein. Eine dritte und kurze Reizung des Vagus verursachte wieder pünktlich eine von Beschleunigung gefolgte Hemmung. Diese Figur liefert also den

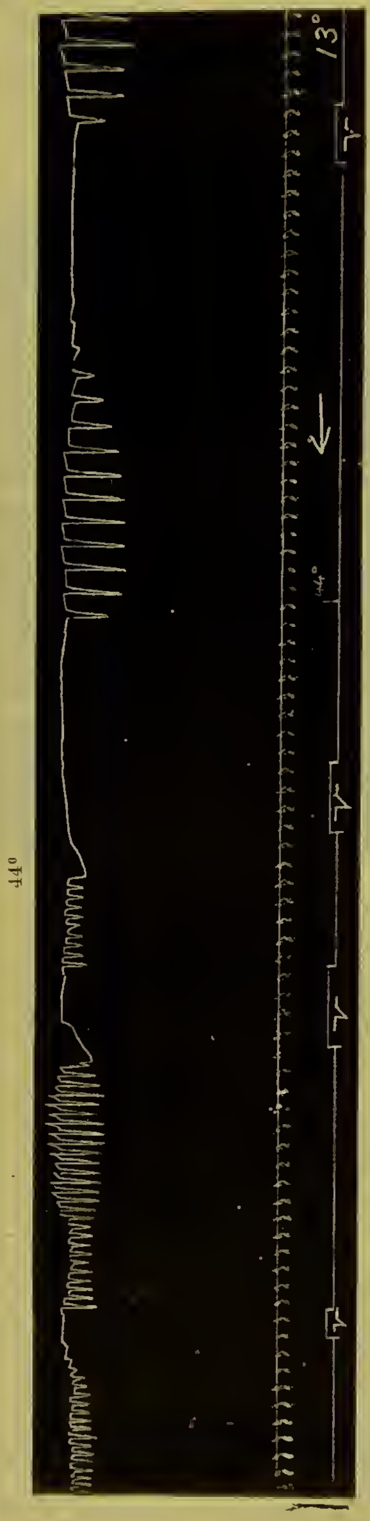

Fig. 9.

Gleiches Herz wie in Fig. 7 u. 8 benutzt. Nur Vorhofkurve wiedergegeben. Für Vagus gleiche Reizstärke wie in diesen Figuren. Lösung von 38° war durch eine solche von Lufttemperatur (13°) ersetzt worden. Reizung des Vagus ist erfolgreich. Bei »44°« wurde eine Lösung (44°) eingeleitet. Stillstand trat ein, Reizung des Vagus verursachte Wiederaufnahme der Schläge. Eine zweite Reizung im Verlauf dieser Kontraktionen (Herz bei 44°), verursachte vollständigen, von Beschleunigung gefolgten Stillstand. Eine dritte Reizung ergab das gleiche Resultat.

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dingende chemische Prozeß). Wird nämlich diese Frequenzänderung auf andere Art hervorgebracht (wie bei dem Herzen des obigen Versuchs, welches zuerst bei Lufttemperatur einen sehr langsamen Rhythmus hat und dann, nachdem es eine Reihe von Temperaturänderungen erlitten hat, eine raschere Schlagfolge annimmt und diese auch bei der ursprünglichen Lufttemperatur beibehält), so antwortet der Hemmungsmechanismus vielleicht in derselben Weise wie bei Steigerung der Herztemperatur.

sicheren Beweis dafür, daß, selbst wenn das Herz in eine Salzlösung von 44° C getaucht war, doch sowohl die Hemmungs- wie auch die Beschleunigungsnerven noch imstande waren, ihre charakteristischen Wirkungen hervorzubringen. Beim Ventrikel war schon lange Stillstand eingetreten. Es ist also sehr zweifelhaft, ob überhaupt die bei der Vorhofkurve so klar zutage getretenen Wirkungen in Kurven entdeckt worden wären, die nur mittels eines einfachen, am Herzen in situ befestigten Hebels registriert worden sind.

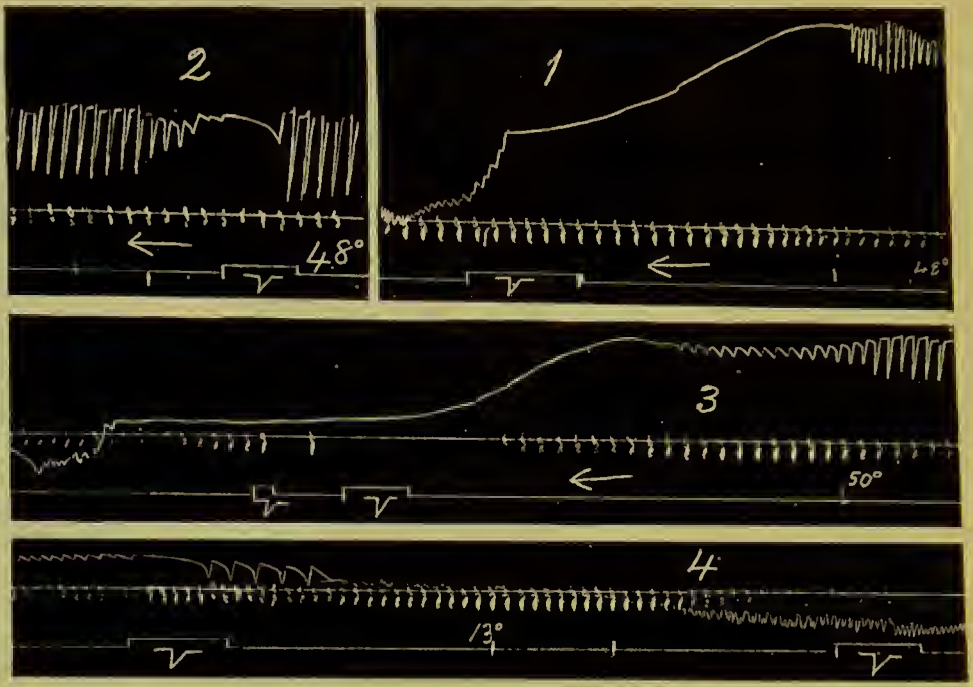

Fig. 10.

Gleiches Froschherz und Reizstärke wie in Fig. 7—9. Nur Vorhofkurve abgebildet. In Nr. 1 trat eine Salzlösung von 48° an Stelle der von 44°. Stillstand und wahrscheinlich teilweise Wärmelähmung trat ein. Reizung des Vagus verursachte Wiederaufnahme der Kontraktionen (von den Sympathicusfasern herrührend). In Nr. 2 verursachte Reizung des Vagus (Herz noch in Lösung von 48°) ersichtlichen Hemmungseffekt. In Nr. 3 Lösung von 50° eingeleitet. Stillstand und teilweise Wärmelähmung erfolgte, zwei Vagusreizungen blieben wirkungslos. Nach Abkühlung der Lösung wurden die Schläge wieder aufgenommen, aber Vagusreizung (Nr. 4) brachte, wenn überhaupt nur geringe Wirkung hervor. Lösung von Zimmertemperatur (13°) wurde eingeleitet, und nun verursachte Vagusreizung Stillstand, von Beschleunigung gefolgt.

In Fig. 10 Nr. 1 wurde die Lösung von 44° durch eine von 48° ersetzt. Stillstand trat ein, von einer großen Steigerung im Tonus gefolgt — zweifellos in Verbindung mit einem gewissen Grad von Wärmestarre.

Reizung des Vagus verursachte weiteres Ansteigen des Tonus mit rapiden, während eines Intervalls von 66 Sekunden rasch an Kraft zunehmenden Ausschlägen (nicht abgebildet — zwischen Nr. 1 und 2). — Man könnte wiederum annehmen, daß der Vagus bei dieser Temperatur seine hemmende Wirkung verloren hätte. Nr. 2 zeigt, daß dies nicht der Fall ist. Der Vorhof schlug in der Salzlösung (48⁰) noch ganz gut, und Reizung des Vagus verursachte einen Stillstand, ausgesprochener noch als der in Fig. 7 bei 13⁰ und über eine mindestens ebenso große oder noch größere Anzahl von Schlägen sich erstreckend. In Fig. 10 (Nr. 3) wurde am bezeichneten Punkt eine Salzlösung von 50⁰ C eingeleitet, es trat Stillstand ein, von einer Zunahme im Tonus gefolgt (teilweise Wärmestarre).

Reizung des Vagus mit der anfänglichen Reizstärke blieb wirkungslos. Als aber das Herz auf 13⁰ abgekühlt wurde (Nr. 4), verursachte der Vagus wiederum Stillstand.

Fig. 11 weist einen anderen Unterschied auf, der bei einfacheren Aufzeichnungsmethoden bestimmt vermißt würde. Die Ventrikel- und Vorhofkontraktionen eines Frosches wurden zuerst bei Lufttemperatur (13,1⁰ C) registriert. Vagusreizung mit 100 mm Rollenabstand verursachte deutliche Hemmung des Vorhofs. Jedoch setzten die Schläge nicht vollständig aus. Beim Ventrikel war keine besondere Hemmungswirkung sichtbar; nur ein einziger Schlag fehlte, und die übrigen Schläge waren ein wenig geschwächt. Bei 24,2⁰ C ist in der Vorhofkurve das Verschwinden der Schläge, infolge der Vagusreizung bei gleichem Rollenabstand, viel deutlicher und daher die Hemmung des Ventrikels viel ausgesprochener. Bei der höheren Temperatur würden während der Hemmungsdauer mehr als zweimal so viel Vorhofschläge erfolgt sein als bei der tieferen Temperatur. Nehmen wir an, daß mit irgendeiner einfachen Registriermethode, die den Ventrikelschlag hauptsächlich oder allein verzeichnete, die Reizstärke bestimmt werden sollte, bei der deutlicher Stillstand eintritt. Es ist sofort klar, daß man dann einen stärkeren Reiz als den in Fig. 11 bei Lufttemperatur angewendeten, dazu gewählt hätte; einen Reiz, der eben eine größere Hemmung des Vorhofschlages hervorgebracht hätte als der dazu völlig genügende, von dem wir ausgingen. So hätte eine Steigerung der Hemmungs-

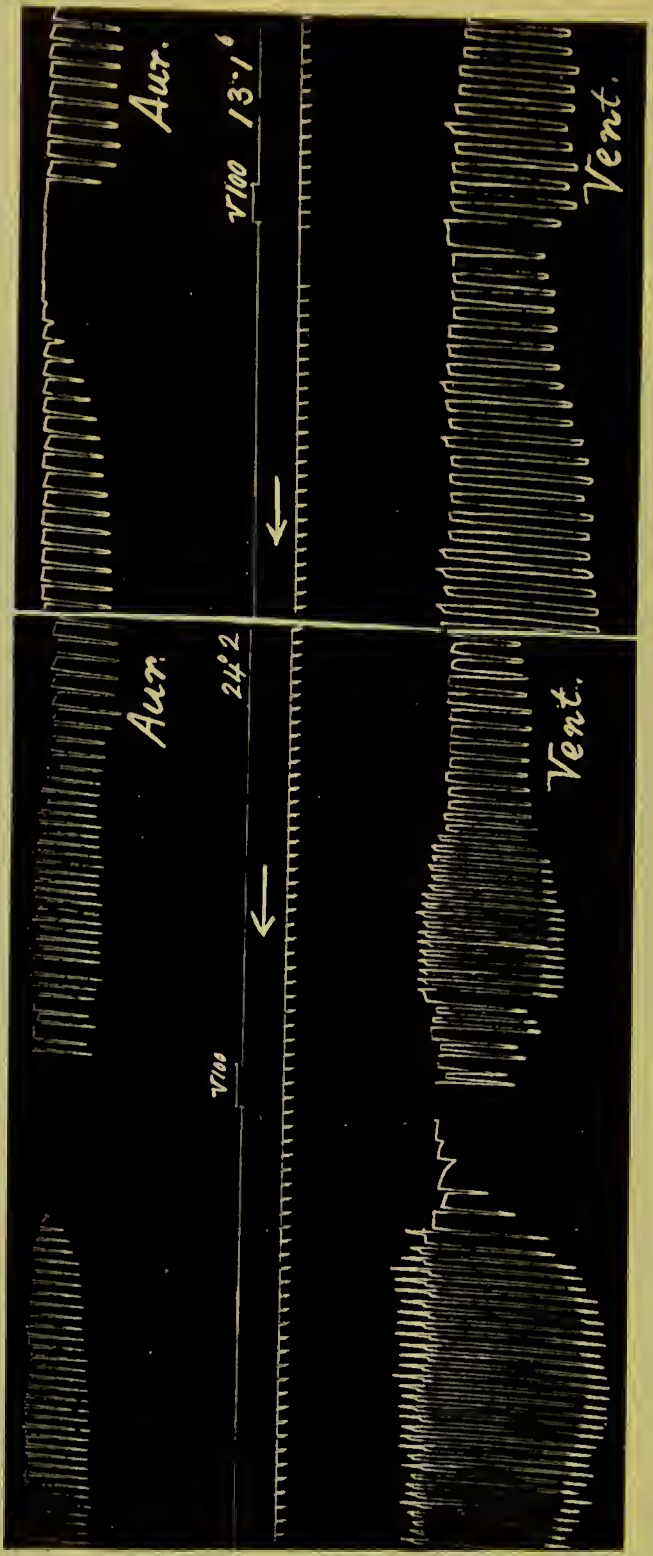

Fig. 11.

Froschherz. — Vorhof- und Ventrikel. — Mit Herz bei Lufttemperatur (13,1°) verursachte Vagusreizung (V. 100) eine gut erkennbare Hemmungswirkung auf den Vorhof, aber auf den Ventrikel nur eine geringe. Mit Herz bei 24,2° war, bei gleicher Stärke und Dauer, die Reizwirkung auf den Ventrikel viel deutlicher.

wirkung des Vagus bei der höheren Temperatur leicht vermißt werden können, weil die Reizstärke zu sehr den Schwellenwert überschritten hätte.

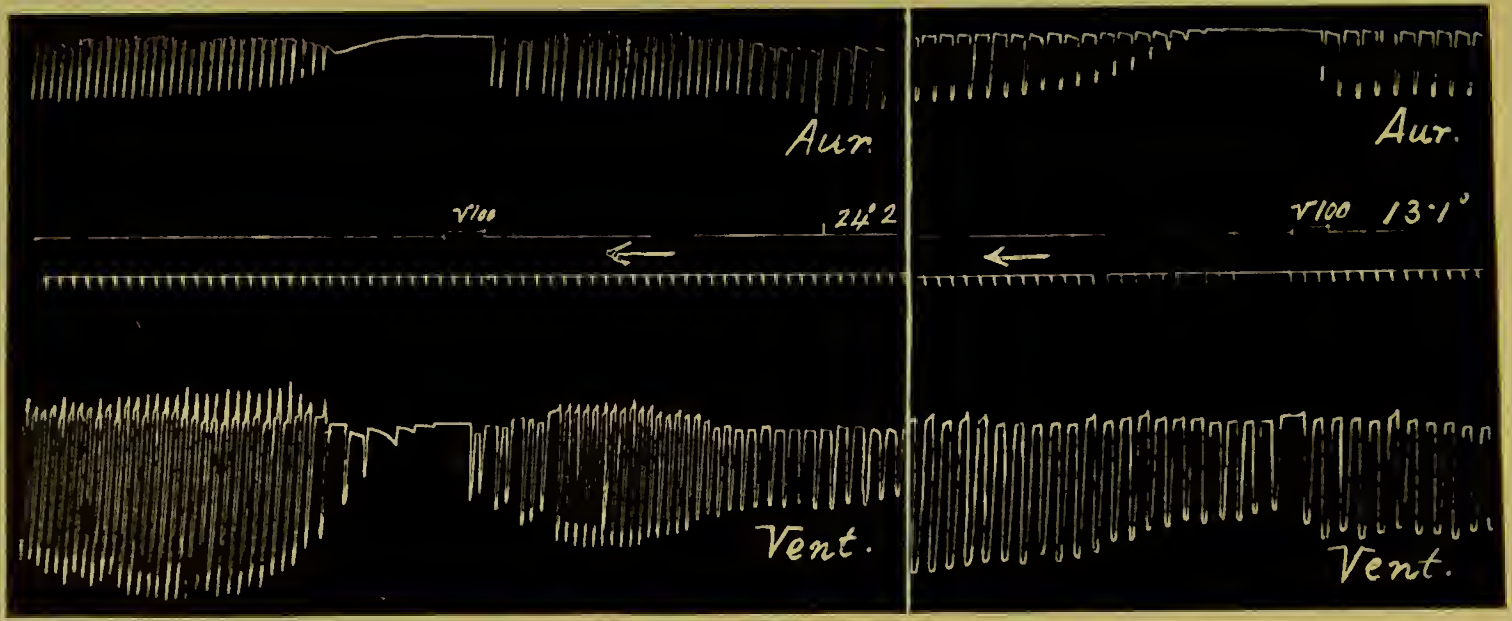

Fig. II.

## III.

## Versuche mit dem Herzen in situ.

Der letzte Punkt, den ich besprechen will, ist der von Pretschistenskaja und andern Forschern gemachte Einwand, daß man Resultate, die mit Gaskells Methode an nicht ernährten Herzen erhalten wurden, nicht auf normal ernährte Herzen anwenden könne, deren Kontraktionen registriert werden unter Bedingungen, die mehr »physiologisch« sind. Wenngleich es ganz unwahrscheinlich ist, daß an dem Froschherzen bei Versuchen von mäßiger Dauer qualitativ verschiedene Resultate erzielt werden sollten, je nachdem das Herz einfach in Flüssigkeit eingetaucht (in ein relativ großes Bad aus Salzlösung) oder mit fremdartigem Blut gefüllt wird, oder ob es in situ bleiben darf, mit dem eigenen Blute gefüllt, machte ich immerhin eine Reihe von Kontrollbeobachtungen an dem Herzen in situ bei intakter Zirkulation. Dieselben wurden schon in der früheren Abhandlung erwähnt; da aber damals kein Platz war für die Wiedergabe einiger Kurven, sollen hier die Resultate durch Fig. 12—14 erläutert werden.

»Es war nicht möglich, die Klammer zu benutzen; das Erwärmen bzw. Abkühlen erfolgte in der Weise, daß man Wasser durch ein kleines gläsernes Schlangenrohr leitete, in dessen Mitte sich das Herz befand. Von der Herzspitze lief ein Faden zu dem Schreibhebel. Die Reibung des Herzens an den Rohrwindungen wurde durch Ölen der letzteren vermindert.« Natürlich war die Temperatur des Herzens niemals gleich der des Wasserstroms. Wenn auch, meiner Meinung nach, die Kurven für den Zweck dieser Untersuchung sehr weit hinter denen zurückstehen, die man mit Gaskells Methode erhält, so lassen sie doch, denke ich, keinen Zweifel darüber, daß die mit dem Herzen in situ erzielten Resultate qualitativ die nämlichen sind wie bei der Suspensionsmethode.

Fig. 12 Nr. 1 zeigt die Wirkung einer Vagusreizung beim Frosch, mit Herz bei Lufttemperatur. In Nr. 2 wurde der Vagus gereizt während Wasser von 25° und in Nr. 3 8° C durch das Rohr geleitet wurde. Der

Rollenabstand war stets der gleiche (120 mm). Bei 25° trat Verlangsamung und dann anscheinend völliger Stillstand ein; aber bei den beiden anderen Temperaturen konnte kein Hemmungseffekt gesehen werden. Ob bei 13° und bei 8° die Kraft d. Vorhofschlages wirklich unberührt blieb, kann man unmöglich bei dieser Registriermethode sagen. Es könnte vielleicht eine ebenso große Hemmung der Vorhoftätigkeit wie in Fig. 11 bei Lufttemperatur vorhanden gewesen sein, würde sich aber doch kaum auf der Kurve ausgedrückt haben, wofern nicht die Leitung der Impulse zum Ventrikel durch den Vagus beeinträchtigt wäre.

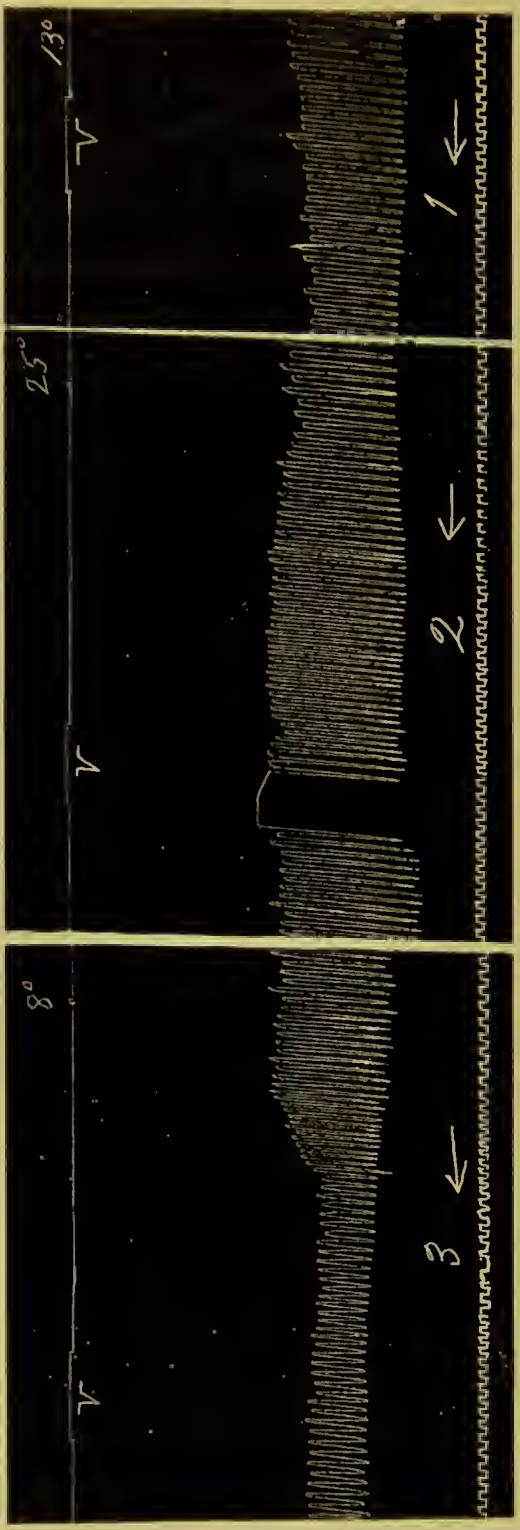

Fig. 12.

Froschherz in situ. — Zeitangabe: Sekunden. — Bei Durchströmung mit Wasser von 13° blieb (bei der verwendeten Reizstärke) die Vagusreizung erfolglos (Nr. 1). — Wasser von 25°, gleiche Reizstärke: Reizung verursacht deutliche Hemmung (Nr. 2). — Mit Wasser von 8° (Nr. 3) zeigte sich kein bemerkenswerter Erfolg bei einer Reizung des Vagus mit gleicher Reizstärke.

Fig. 13 und 14 geben Kurvenabschnitte vom Herzen eines anderen Frosches (mit Schlangenrohr). Der Vagus wurde während des ganzen Versuchs mit dem gleichen Rollenabstand gereizt, ausgenommen wo anderes bemerkt ist. Nr. 1 zeigt geringe Hemmung bei Lufttemperatur, aber entschieden weniger als bei Anwärmung des Rohrs mit Wasser von 33° C (Nr. 3) oder 36° C (Nr. 4). In Beobachtung Nr. 5 wurde am angegebenen Punkt Wasser von 40° C durch die Röhre geleitet und diese Durchströmung wurde in der Zeit zwischen Nr. 5 bis Nr. 6

(96 Sekunden) fortgesetzt. Bei dieser Wassertemperatur war die Vagusreizung ganz gut wirksam (Nr. 6). Bei Durchleitung von Wasser mit 45° C,

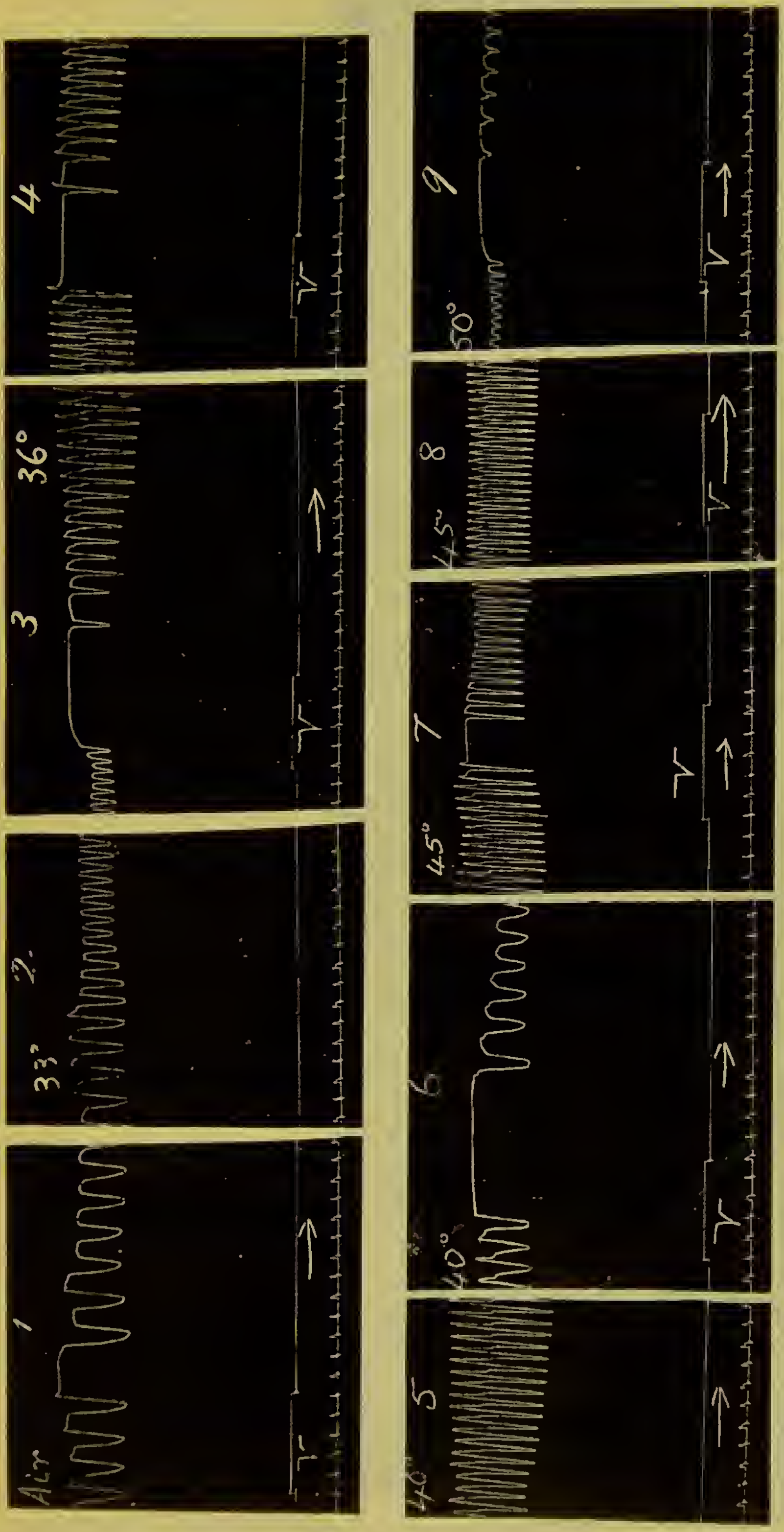

Fig. 13.

Froschherz in situ. — Gleiche Reizstärke für den Vagus angewendet (120 mm Rollenabstand) in allen Beispielen, ausgenommen Nr. 9, wo ein etwas stärkerer Reiz benutzt wurde. In Nr. 1 war das Herz in Lufttemperatur. In Nr. 2 begann Wasser von 33° die Röhre zu durchfließen und ebenso in Nr. 3. Bei »36°« wurde Wasser von 36° durchgeleitet und desgleichen bei Nr. 4. In Nr. 5, beim angegebenen Punkt, begann Wasser von 40° die Röhre zu durchfließen, desgleichen in Nr. 6. Hierauf wurde mit Wasser von 45° gewechselt und damit in Nr. 7 fortgefahren. Während Nr. 9: Wasser von 50° durch geleitet.

(welches 66 Sekunden lang vor Beginn der in Nr. 7 wiedergegebenen Zeichnung das Rohr durchfloß) wurde durch Vagusreizung noch Stillstand er-

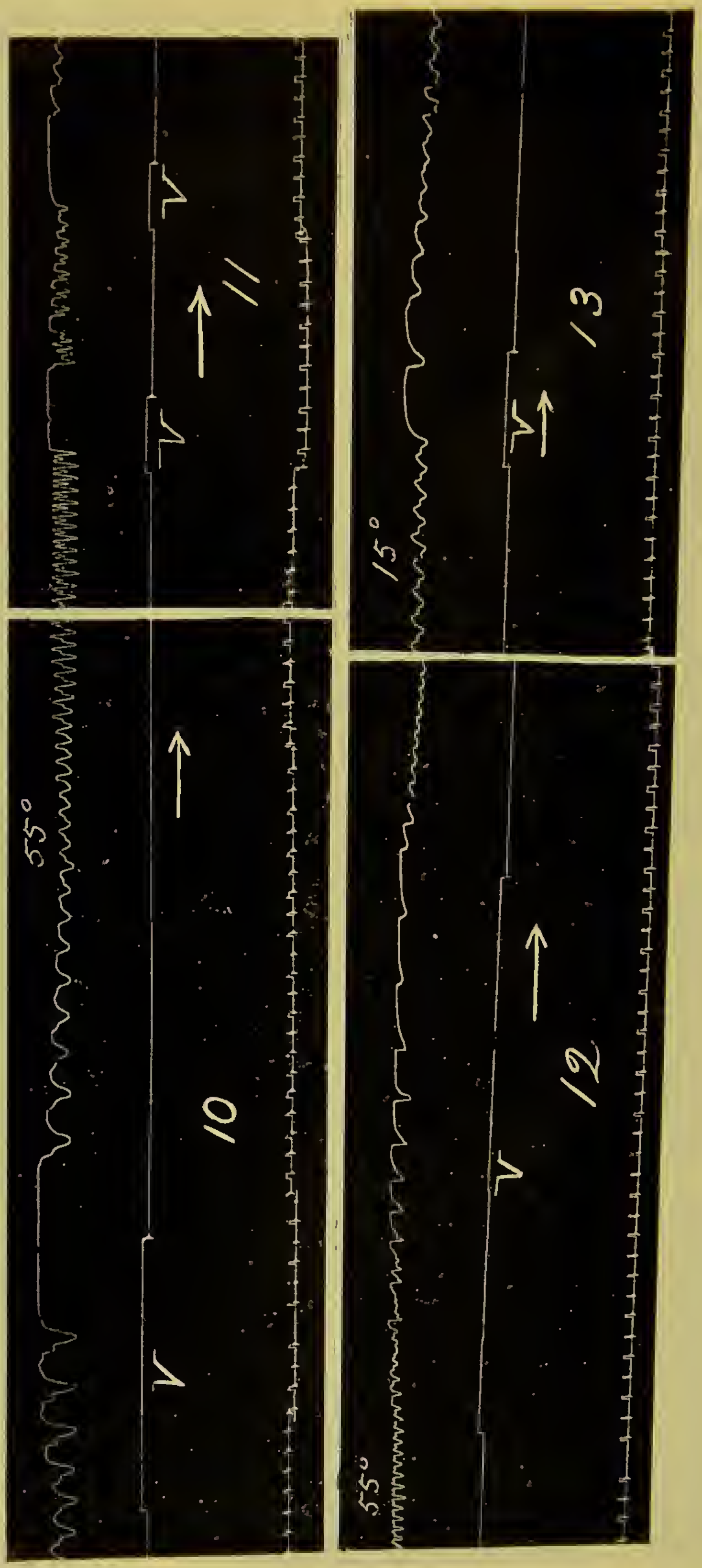

Fig. 14.

Stellt die Fortsetzung des in Fig. 13 begonnenen Versuchs dar. In Nr. 10 wurde der Vagus mit der in Nr. 9 (Fig. 13) benutzten erhöhten Reizstärke erregt, während Wasser von 50° die Röhre durchfloß. Bei »55°« wurde mit Wasser von 55° gewechselt und während dies die Röhre durchfloß, wurde der Vagus zweimal gereizt (mit gesteigerter Reizstärke), in Nr. 11 und später in Nr. 12. In Nr. 13 wurde Wasser von 15° durch die Röhre geleitet und der Vagus mit der erhöhten Reizstärke gereizt.

zielt. Zwischen 7 und 8 verging ein Zeitraum von 3 Minuten 10 Sekunden; während dieser Zeit strömte Wasser von 45° durch das Rohr.

Reizung des Vagus in der bisher benutzten Stärke war nun wirkungslos. Eine weitere Reizung, 28 Sekunden nach Schluß von Nr. 8, war gleichfalls ohne Erfolg, wie auch zwei weitere Reizungen zwei Minuten später. Diese letzten drei Reizungen sind in der Figur nicht wiedergegeben. Der Wasserstrom in dem Rohr wurde dabei dauernd auf 45° gehalten. Nunmehr wurde Wasser von 50° C eingeleitet. Nach Verlauf von 40 Sekunden blieb eine Reizung des Vagus bei dem früheren Rollenabstand wiederum wirkungslos. Wasser von gleicher Temperatur wurde noch weitere 1½ Minuten bis gegen Beginn der in Nr. 9 wiedergegebenen Kurve durch das Rohr geleitet. Als nun der Vagus mit einem nur wenig stärkeren Reiz erregt wurde, trat bedeutende Hemmung ein (Beobachtung 9). Wasser von 50° C floß weitere 3 Minuteü durch das Rohr, und eine Reizung des Vagus mit dem verstärkten Reize erzielte eine entschiedene Hemmung (Fig. 14 Nr. 10). Die ursprüngliche Reizstärke hatte dagegen keine Wirkung (nicht abgebildet).

Alsdann wurde Wasser von 55° C eingeleitet, und Reizung des Vagus mit dem verstärkten Reiz war wiederum wirksam (Nr. 11). Selbst nachdem das Wasser von 55° C noch weiter einige Zeit durchgeflossen war, hatte Vagusreizung mit dieser vermehrten Reizstärke noch Erfolg (Nr. 12), obgleich das Herz sehr schwach, jedoch rasch schlug. Die ursprüngliche Reizstärke blieb wirkungslos (in der Figur nicht abgebildet). Wasser von 15° C wurde hierauf 4 Minuten lang durch das Rohr geleitet und der Vagus (Fig. 14 und Nr. 13) mit dem gesteigerten Reiz behandelt. Eine geringe Hemmung wurde erhalten, obgleich die ursprüngliche Reizstärke keine Wirkung erzielte.

Es ist hier klar, daß wir es mit einer dauernden Verminderung der Wirksamkeit des Hemmungsmechanismus zu tun haben, die möglicherweise von einer teilweisen Lähmung der Zellen längs des Hemmungsweges im Herzen infolge hoher Temperatur herrührt. Es muß bemerkt werden, daß die Temperatur der äußeren Schichten des Herzens entschieden höher sein kann als die innere, sobald die Erwärmung auf der Außenseite des Herzens stattfindet, wie dies bei den Versuchen mit dem Schlangenrohr und bei den Versuchen von Clark mit erhaltener Zirkulation der Fall. Es wird daher der Hemmungsnervenbahn eine größere Schädigung zugefügt werden dort, wo sie oberflächlich verläuft, als an dem übrigen Herzen. Diese Möglichkeit ist besonders gegeben in den Versuchen von Clark, bei denen das erwärmende Instrument auf die Grenze von Sinus und rechtem Vorhof aufgesetzt wurde.

Völlige Unerregbarkeit des Vagus jeder Reizstärke gegenüber weist hin auf die Gegenwart eines sehr wirksamen Blocks irgendwo auf dem Wege der Leitungsbahn. Solche Hindernisse wurden in meiner Arbeit, wahrscheinlich aus dem bereits erwähnten Grunde, sehr selten angetroffen und wären auch mit dem Zweck meiner Untersuchung nicht gut zu vereinbaren gewesen.

# A STUDY OF VASOMOTOR REFLEXES ELICITED BY HEAT AND COLD FROM REGIONS DEVOID OF TEMPERATURE SENSIBILITY (IN AN UNUSUAL CASE OF POST-TYPHOIDAL NEURITIS)*

G. N. STEWART, M.D., AND WALTER B. LAFFER, M.D.
CLEVELAND

This study was undertaken with two objects: one clinical, the other physiological. From both aspects the results obtained seem to be of sufficient interest and utility to warrant their publication. The clinical object was primarily to throw light, if possible, on the diagnosis of a case which seemed to present considerable difficulty and concerning which very different conclusions had been arrived at by competent clinical observers. The physiological object was to contribute something to the solution of the question whether the afferent fibers through which the vasomotor reflexes induced by the application to the skin of heat and cold are identical with the fibers concerned in the corresponding sensations, a question which, so far as we are aware, has not received a definite answer. It is, of course, known that actual consciousness of the sensations is not necessary for the development of the vasomotor reactions, since they occur also in sleep. But we do not know whether a lesion, especially a peripheral lesion, which completely blocks the transmission of impulses along the nerves of temperature sensation, or at least prevents them from rising to the threshold value necessary for the production of sensation, blocks at the same time all the afferent vasomotor impulses set up by heat or cold. An answer to this question is not only of physiological interest, but also might, in certain cases, prove of diagnostic importance, particularly in affording a basis for an objective test of the existence and possibly for an objective measure of the acuity of temperature sensibility.

The vasomotor reflexes in this study were investigated by means of the method of measuring the blood-flow, previously described by one of us.[1] The reflexes elicited in one hand when the other hand or a portion of it is placed in cold or warm water, that is to say, the contralateral vaso-motor reflexes, were preferred for this study to the ipsolateral reflexes induced in the hand immersed in the hot or cold water, since it is commonly stated that even in the absence of connection with the central nervous system the blood-vessels quickly regain the power of responding to local changes of temperature through the intervention of a local

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*From the City Hospital and the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University.

TABLE 1.—RESULTS OF CALORIMETER TESTS ON R. E.

April 26, 1912. R. E. Hands in bath at 1:51½ p. m. Hands in calorimeters at 2:03 p. m. 3,015 c.c. water in each. Examination made in hospital.

| Time | R. | L. | Notes |
|---|---|---|---|
| 2.02 | 31.00 | 30.76 | |
| 2.04 | 30.91 | 30.69 | Room 24.5 C. |
| 2.05 | 30.90 | 30.67 | |
| 2.06 | 30.88 | 30.65 | |
| 2.07 | 30.86 | 30.63 | |
| 2.08 | 30.84 | 30.63 | |
| 2.10 | 30.82 | 30.61 | Room 24.3. |
| 2.11 | 30.80 | 30.59 | |
| 2.13 | 30.78 | 30.57 | |
| 2.15 | 30.77 | 30.555 | Room 24.25 C. |
| 2.17 | 30.76 | 30.545 | |
| 2.19 | 30.75 | 30.55 | |
| 2.21 | 30.77 | 30.59 | Room 24.2 C. |
| 2.22 | 30.775 | 30.605 | |
| 2.23 | 30.78 | 30.61 | |
| 2.24 | 30.79 | 30.635 | |
| 2.25 | 30.795 | 30.655 | |
| 2.26 | 30.80 | 30.675 | |
| 2.27 | 30.805 | 30.70 | |
| 2.28 | 30.81 | 30.725 | Room 24.0 C. |
| 2.29 | 30.82 | 30.73 | |
| 2.30 | 30.83 | 30.745 | |
| 2.31 | 30.84 | 30.765 | |
| 2.32 | 30.86 | 30.79 | |
| 2.33 | 30.875 | 30.825 | |
| 2.34 | 30.885 | 30.835 | Right hand and wrist put in water at 43 C. Feels it on wrist. |
| 2.35 | ...... | 30.85 | |
| 2.36 | ...... | 30.865 | |
| 2.37 | ...... | 30.875 | |
| 2.38 | ...... | 30.895 | |
| 2.39 | ...... | 30.915 | |
| 2.40 | ...... | 30.93 | |
| 2.41 | ...... | 30.965 | |
| 2.42 | | 30.99 | |
| 2.43 | | 31.025 | |
| 2.44 | | 31.05 | Room 24.0 C. |
| 2.45 | | 31.095 | |
| 2.46 | | 31.12 | Here cold water was brought into room and excited his apprehensions. |
| 2.47 | | 31.13 | |
| 2.48 | | 31.15 | At 2.48 right hand and wrist put into water at 8 C. He feels it very cold on wrist, but not at all on fingers. Feels fingers somewhat numb. |
| 2.49 | | 31.17 | |
| 2.50 | | 31.19 | |
| 2.51 | | 31.20 | |
| 2.52 | | 31.205 | |
| 2.53 | | 31.21 | |
| 2.54 | | 31.215 | |
| 2.55 | | 31.215 | Still feels the right wrist very cold. |
| 2.56 | | 31.22 | The cold water is now at 12.5 C. |
| 2.57 | | 31.215 | Room 23.6 C. |
| 2.58 | | 31.225 | |
| 2.59 | | 31.225 | |
| 3.00 | | 31.23 | |
| 3.01 | | 31.235 | |
| 3.02 | | 31.24 | Room 23.6 C. |
| 3.03 | | 31.25 | At 3.03 right hand and wrist put into water at 43 C. The wrist still felt cold for a little time in the warm water. |
| 3.04 | | 31.255 | |
| 3.05 | | 31.27 | |
| 3.06 | | 31.29 | |
| 3.07 | | 31.305 | |
| 3.08 | | 31.32 | |
| 3.09 | | 31.325 | |
| 3.10 | | 31.325 | |
| 3.11 | | 31.325 | |
| 3.12 | | 31.325 | At 3.12 took hand out of calorimeter. |
| 3.25½ | | 31.17 | The right hand is reddish; obviously fair vasodilatation. Temperature of R. 30.395 C. |

Cooling of R. 0.49° in 51½ minutes. Cooling of L. 0.155° in 13½ minutes. Volume of right hand 379 c.c., of left 397 c.c. Rectal temperature 37.05 C.

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[1] Heart, 1911, iii, 33, 76; THE ARCHIVES INT. MED., 1912, ix, 706.

regulating mechanism. Thus Trotter and Davies,[2] who studied the phenomena following the section of cutaneous nerves in themselves, state that "The vasomotor changes which follow nerve-section begin to show a diminution within a few days of the operation, and by the end of six weeks scarcely any abnormality of this function can be detected. It seems probable, therefore, that recovery occurs by some local compensatory mechanism rather than by an active regeneration of the vasomotor nerve fibers."

TABLE 2.—RESULTS OF CALORIMETER TESTS ON R. E.

May 2, 1912, R. E. Hands in bath at 3.15 p. m. Hands in calorimeters at 3.26 p. m. Mouth temperature 36.57 C. Temperature of arterial blood at wrist (say) 36.6 C. Examination made in hospital.

| Time | R | L | Notes | Time | R | Notes |
|---|---|---|---|---|---|---|
| 3.24½ | 31.10 | 31.16 | | 3.45 | 31.235 | At 3.43 left wrist withdrawn. He feels warmth only on middle phalanx of left index finger. Hand kept thus for the rest of the experiment. |
| 3.27 | 31.07 | 31.15 | | 3.46 | 31.27 | |
| 3.28 | 31.09 | 31.16 | Room 25.5 C. | 3.47 | 31.305 | |
| 3.29 | 31.095 | 31.17 | | 3.48 | 31.345 | |
| 3.30 | 31.105 | 31.185 | | 3.49 | 31.395 | Room 24.9 C. |
| 3.31 | 31.105 | 31.20 | Room 25.4 C. | 3.50 | 31.41 | At 3.50 left hand put in cold water at 8.3 C. Wrist not immersed. Only feels cold on middle phalanx of index finger. |
| 3.32 | 31.11 | 31.21 | | 3.51 | 31.45 | |
| 3.33 | 31.11 | 31.225 | | | | |
| 3.34 | 31.12 | 31.225 | | | | |
| 3.35 | 31.125 | 31.23 | | 3.52 | 31.47 | Room 25.0 C. |
| 3.36 | 31.125 | 31.23 | | 3.53 | 31.48 | Still feels cold intensely on middle phalanx of index. The cold water is now at 10.4 C. |
| 3.37 | 31.135 | 31.225 | At 3.37 left hand put in water at 43 C. Room 25.4 C. He feels warmth on wrist. | 3.54 | 31.495 | |
| 3.38 | 31.13 | ...... | | | | |
| | | | | 3.55 | 31.505 | Still complains of cold in this phalanx. |
| | | | | 3.56 | 31.52 | |
| 3.40 | 31.14 | ...... | At 3.40 withdrew wrist, dried it, and put in cloth to keep it from vessel. Now feels warmth only on middle phalanx of left index finger. At 3.42 left wrist immersed. He feels it warm. | 3.57 | 31.525 | |
| 3.41 | 31.15 | ...... | | 3.58 | 31.535 | Hands out at 3.58. |
| 3.42 | 31.155 | ...... | | 4.09 | 31.425 | Temp. of L. is now 30.95 C. |
| 3.43 | 31.175 | ...... | | | | |
| 3.44 | 31.195 | ...... | | | | |

Cooling of calorimeters R. 0.11° in 11 minutes. L. 0.275° in 32 minutes. Volume of right hand, 361 c.c.; left hand, 385 c.c. Pulse 76 (half reclining).

## REPORT OF CASE

*History.*—The patient first came under observation of one of us in 1898, when he showed very marked atrophy of the small muscles of the hands, with less atrophy of the extensor group of the forearms and legs. He was not under close observation then until 1908, when the following history and condition were elicited:

R. E., white, aged 44 years, widower, laborer.

*Family History.*—Father died at the age of 57 of "paralysis of the brain." Mother died at the age of 30 of "childbirth." Three sisters and three brothers dead; four died when very small; one died of typhoid fever and one died as the result of an accident. One uncle died of tuberculosis. Grandfather died of "liver trouble."

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[2] Trotter and Davies: Jour. Physiol., 1909, xxxviii, 188.

*Previous History.*—The patient had scarlet fever, measles, and mumps when small; had typhoid fever twenty-four years ago (in 1884), rheumatism in left shoulder two or three years previous to examination, and again a slight recurrence in the right leg during the previous seven months. The patient denies ever having had any venereal disease; had much catarrh sixteen years previously; could not smell anything unless very strong for the previous ten years. Had cough and expectoration for the previous five or six years. Tubercle bacilli were found in the sputum twice. He had pleurisy several times two or three years prior to examination, and was short of breath for five or six years; ankles swollen some six years previously; appetite only fair, some vomiting after eating for the previous year or so; pain in stomach at times—cramp-like, yet not very sharp; bowels regular; no incontinence of feces; some incontinence of urine eight or nine months previously that lasted for three months; had had no incontinence lately, no slowness in urination, and no change in sexual function. The patient experienced unsteadiness in the dark for twenty years; also when he closed his eyes to wash his face. There was some double vision for previous six years; no paresthesia. Wasting of hands, arms and legs, first noticed about the knees followed typhoid fever twenty-four years previously. He noted loss of sensation in hands, then in feet three or four years after he noticed the muscular wasting. He had fourteen operations for empyema of the frontal sinuses. When he had typhoid fever he had pain in the right groin; no bed sores; hair came out; noted much muscular wasting while sick; could not walk for three months after getting over his typhoid; had no pain in legs or arms at that time. Wasting of muscles was very marked three years after typhoid and about this time he noted loss of sensation, first in the lower legs, then in the feet, and then in the arms and hands. Feet numb; no stinging or burning. At this time the urine and sputum showed nothing abnormal.

*Physical Examination.*—This showed scars over the frontal sinuses from repeated operation, which interfered with the action of the frontal muscles; no pupillary reaction to ordinary light; no nystagmus; some contraction of the field of vision in all diameters; jaw-jerk not present; no glandular enlargement, no changes in the bones or joints; no scars or pigmentation of the skin.

*Muscular System.*—Quite marked atrophy of the trapezii, supraspinati and also of the serratus magnus on the right side; some wasting of the serratus magnus on the left. Both erector spinae muscles rather small and wasted. Considerable strength in the biceps, triceps and deltoid on both sides; also of the supinators and pronators; very marked atrophy of all of the small muscles of the hands and extensors and flexors of the forearm. Ulnar border of forearms concave due to muscular atrophy. Marked atrophy of the thenar and hypothenar eminences. He could not "make a fist" with either hand and had practically no grip. The face had a somewhat mask-like appearance, probably due to the sinus operations. He had a double partial ptosis (due to a sinus operations?); was able to shut both eyes; there seemed to be no paralysis of the facial muscles and the orbicularis oris was strong. The muscles of the soft palate and tongue were normal. The voice was husky, more like a voice of weakness than of paralysis. Platysma myoides and sternocleidomastoid and other muscles of the neck were much atrophied. The pectoral muscles showed good volume. The abdominal muscles were greatly weakened so that he could not bring himself into a sitting position without the aid of his arms. The atrophy was more marked on the left side. There was considerable wasting of all groups of muscles of the thigh, especially the abductors; marked atrophy of the peroneal groups, causing toe-drop; fairly good power in the calf muscles; extensors and flexors practically without power; marked wasting of the interossei; had some flat-foot.

*Reflexes.*—Bicipital, elbow and wrist-jerks all absent. Abdominal reflex present at all levels; cremasteric absent. Knee-jerks and Achilles-jerk absent on

May 8, 1912. R. E. He says he feels better and is in better spirits than at the two previous examinations, partly due to the fact that his wife accompanied him; also to the expectation of some pecuniary reward. Examination made in laboratory. Hands in bath at 1.43 p. m., in calorimeters at 1.53 p. m.; 3,015 c.c. in each. Mouth temp. 36.95 C.

FIRST PART OF TEST

| Time | R | L | Notes | Time | R | L | Notes |
|---|---|---|---|---|---|---|---|
| 1.52 | 30.995 | 30.97 | Room 22.2 C. | 2.01 | 31.01 | 31.04 | |
| 1.54 | 30.935 | 30.925 | | 2.02 | 31.025 | 31.05 | Room 22.9 C. |
| 1.55 | 30.935 | 30.93 | | 2.03 | 31.04 | 31.065 | |
| 1.56 | 30.935 | 30.93 | | 2.04 | 31.065 | 31.095 | |
| 1.57 | 30.96 | 30.955 | Room 22.7 C. | 2.05 | 31.095 | 31.125 | Room 23.0 C. |
| 1.58 | 30.98 | 30.99 | | 2.06 | 31.11 | 31.14 | |
| 1.59 | 30.995 | 31.01 | | 2.07 | 31.13 | 31.165 | |
| 2.00 | 31.00 | 31.025 | | 2.08 | 31.15 | 31.20 | |

SECOND PART OF TEST

| Time | L | Notes | Time | L | Notes |
|---|---|---|---|---|---|
| 2.09 | 31.205 | At 2.08 right hand put in water at 43 C. Wrist not immersed. He does not feel warmth at all. | 2.39 | 31.71 | |
| 2.10 | 31.22 | | 2.40 | 31.725 | At 2.40½ right hand put in water at 8 degrees. Wrist carefully dried and not immersed. He did not feel the cold at all. |
| 2.11 | 31.23 | | 2.41 | 31.735 | |
| 2.12 | 31.235 | Room 22.8 C. | 2.42 | 31.74 | |
| 2.13 | 31.25 | The warm water is now at 41 C. | 2.43 | 31.75 | |
| 2.14 | 31.26 | | 2.44 | 31.765 | Room 22.7. |
| 2.15 | 31.295 | Room 22.95 C. | 2.45 | 31.78 | |
| 2.16 | 31.32 | | 2.46 | 31.795 | |
| 2.17 | 31.325 | | 2.47 | 31.805 | The cold water is now at 9.7°. |
| 2.18 | 31.33 | | 2.48 | 31.81 | |
| 2.19 | 31.34 | | 2.49 | 31.825 | |
| 2.20 | 31.35 | Room 22.9 C. | 2.50 | 31.83 | Room 22.6. |
| 2.21 | 31.36 | | 2.51 | 31.835 | |
| 2.22 | 31.38 | | 2.52 | 31.835 | |
| 2.23 | 31.405 | | 2.53 | 31.835 | |
| 2.24 | 31.425 | | 2.54 | 31.84 | At 2.54 right hand, including wrist, put into water at 43°. He feels the wrist warm. |
| 2.25 | 31.435 | Room 22.7 C. | 2.55 | 31.84 | |
| 2.26 | 31.45 | | 2.56 | 31.84 | |
| 2.27 | 31.47 | | 2.57 | 31.84 | Room 22.8. |
| 2.28 | 31.495 | At 2.28 right hand, including wrist, immersed in water at 43 C. Feels it warm on wrist. | 2.58 | 31.84 | |
| 2.29 | 31.50 | | 2.59 | 31.845 | |
| 2.30 | 31.525 | Room 22.9 C. | 3.00 | 31.845 | |
| 2.31 | 31.53 | | 3.01 | 31.85 | |
| 2.32 | 31.54 | | 3.02 | 31.855 | |
| 2.33 | 31.565 | | 3.03 | 31.855 | |
| 2.34 | 31.59 | Room 22.7 C. | 3.05 | 31.85 | At 3.05 hand out of calorimeter. Temp. of R. 30.39. |
| 2.35 | 31.625 | | 3.13 | 31.74 | |
| 2.36 | 31.635 | | | | |
| 2.37 | 31.65 | | | | |
| 2.38 | 31.685 | Room 22.8 C. | | | |

Cooling of calorimeter R. 0.76 degrees in 65 minutes; cooling of calorimeter L. 0.11 degrees in 8 minutes. Volume of right hand 403 c.c. Volume of left hand, 421 c.c.

both sides. No plantar reflex; no Babinski, Oppenheim or Gordon reflexes were present on the left side. There was a slight plantar reflex on the right side involving but the four small toes, and with this stimulus the quadriceps contracted although he felt nothing. No Babinski, Oppenheim or Gordon present on the right side; gluteal reflexes present; no enlargement or tenderness of the nerve trunks found.

*Sensory Symptoms.*—The patient was anesthetic to all forms of sensation over the frontal region laterally to the line of the outer canthus of the eyes, probably due to nerve lesions caused by sinus operations. No anesthesia of the rest of the face. No anesthesia of the neck or trunk. Toes, feet, and legs were anesthetic to all forms of sensation to above the knees. On the right side the line of anesthesia is just at the upper border of the patella and running around to one inch higher on the posterior surface. On the left leg the line of anesthesia was an inch above the patella in front, curving downward a little on each side, then curving up posteriorly to the upper part of the popliteal space. Analgesia extended to about two inches higher than anesthesia to touch in front and slightly lower posteriorly. Deep pressure sense was absent in the legs.

Left arm, hand and fingers were anesthetic to all forms of sensation from a line about three-fourths inch above elbow-joint.

Right arm, hand, and fingers were anesthetic to all forms of sensation, including deep pressure, below a line about one and one-half inches above the condyle of the humerus, extending a little higher on the anterior surface.

Stereognostic sense was gone in the hands; could not tell position of fingers or hand.

Patient stood with knees over-extended and walked with steppage gait, throwing right foot farther forward, however, than left. Toes had a tendency to drag, due to drop-foot.

Lumbar puncture showed low pressure, clear fluid; twelve cells, mostly lymphocytes, to the c.mm. Urine showed nothing abnormal.

April 13, 1909, the patient showed slight nystagmus; contrary to what was previously observed pupils equal and reacted to light and accommodation. Seemingly some weakness of the labial muscles and cheek muscles. Tongue showed wrinkling and thinning, seemingly slight atrophy, equal on both sides. Some difficulty in swallowing owing to the food coming out of the nose.

He said he could not walk down hill, but must run down owing to weakness of the extensors of the legs. At this time both patellar and both plantar reflexes were absent.

April, 1912, blood showed a negative Wassermann reaction.

May, 1912, patient again came under observation at the Cleveland City Hospital and a few additional facts in regard to the history may be added.

The patient stated that he was in a sanatorium for tuberculosis seven years previously and was discharged as seemingly cured after nine months' stay. He gave no history of lightning pains or gastric crises. He still experienced considerable difficulty in walking. He had some trouble at times in controlling the bladder and had several involuntary bowel movements, but as a rule he had no trouble in controlling the sphincters of bladder and bowel.

He said his feet felt cold and numb at times, but he had no other subjective sensory symptoms.

He had lately married for the fourth time. History of his marital relations showed nothing suggestive of a syphilitic infection.

He believed that the strength in his hands and feet was slowly improving, which seems to be very certain judging from the records of former examinations. The patient said his hands and feet always felt cold to other people when he, himself, could tell no difference.

Examination of the chest showed evidence of a quiescent tuberculosis at the right apex. Cardiovascular system shows nothing abnormal, although heart sounds seem quite faint. No murmurs present.

## TABLE 4.—RESULTS OF CALORIMETER TEST ON R. E.

May 18, 1912. R. E. He came from his home to the City Hospital for this examination. The weather was fairly warm. Hands in bath at 11:48 a. m. Hands in calorimeters at 11.58 a. m. 3.015 c.c. water in each. Mouth temperature, 36.55 C.

### FIRST PART OF TEST

| Time | R. | L. | Notes | Time | R. | L. | Notes |
|---|---|---|---|---|---|---|---|
| 11.57½ | 30.91 | 30.87 | Room 24.8 C. | 12.09 | 30.99 | 30.985 | |
| 11.59 | 30.89 | 30.85 | | 12.10 | 30.995 | 31.00 | |
| 12.00 | 30.90 | 30.86 | | 12.11 | 31.00 | 31.02 | Room 24.8 C. |
| 12.01 | 30.91 | 30.88 | Room 24.9 C. | 12.12 | 31.01 | 31.025 | |
| 12.02 | 30.93 | 30.91 | He saw cold water being prepared with ice. | 12.13 | 31.02 | 31.035 | |
| 12.03 | 30.94 | 30.92 | | 12.14 | 31.035 | 31.045 | Room 24.6 C. |
| 12.04 | 30.94 | 30.925 | Room 24.9 C. | 12.15 | 31,045 | 31.055 | |
| 12.05 | 30.945 | 30.93 | | 12.16 | 31.06 | 31.065 | |
| 12.06 | 30.96 | 30.94 | | 12.17 | 31.065 | 31.08 | At 12.17 right hand (not wrist) put into water at 8.2 C. He did not feel the cold at all. |
| 12.07 | 30.97 | 30.955 | | | | | |
| 12.08 | 30.98 | 30.97 | Room 24.9 C. | | | | |

### SECOND PART OF TEST

| Time | L. | Notes | Time | L. | Notes |
|---|---|---|---|---|---|
| 12.18 | 31.085 | | 12.49 | 31,555 | |
| 12.19 | 31.09 | Room 24.4 C. | 12.50 | 31.575 | At 12.50 immersed right hand over wrist in the cold water. Feels it very cold on wrist. The water is 8.9 C. |
| 12.20 | 31.10 | | 12.51 | 31.58 | |
| 12.21 | 31.11 | Room 24.6 C. | 12.52 | 31.59 | |
| 12.22 | 31.125 | | 12.53 | 31.585 | Room 24.6 C. |
| 12.23 | 31.14 | | 12.54 | 31.60 | |
| 12.24 | 31.155 | Room 24.5 C. | 12.55 | 31.61 | Still feels it quite cold on wrist. |
| 12.25 | 31.17 | | 12.56 | 31.625 | Room 24.6 C. |
| 12.26 | 31.19 | | 12.57 | 31.625 | |
| 12.27 | 31.22 | | 12.58 | 31.625 | |
| 12.28 | 31.23 | Room 24.5 C. | 12.59 | 31.63 | Room 24.7 C. |
| 12.29 | 31.24 | At 12.29 right hand (not wrist) put into water at 43.5 C. Feels nothing. Room 24.7 C. | 1.00 | 31.63 | |
| 12.30 | 31.25 | | 1.01 | 31.635 | |
| 12.31 | 31.25 | | 1.02 | 31.64 | At 1.02 right hand and wrist put into water at 43.4 C. Feels it warm on wrist. Room 24.9 C. |
| 12.32 | 31.27 | The cold water is now 9.7 C. | 1.03 | 31.645 | |
| 12.33 | 31.31 | | 1.04 | 31.65 | |
| 12.34 | 31.325 | Does not feel the warm water. | 1.05 | 31.67 | |
| 12.35 | 31.34 | Room 26.9 C. | 1.06 | 31.69 | Room 24.9 C. |
| 12.36 | 31.365 | | 1.07 | 31.71 | |
| 12.37 | 31.40 | | 1.08 | 31.72 | |
| 12.38 | 31.42 | Room 24.9 C. | 1.09 | 31.725 | |
| 12.39 | 31.425 | | 1.10 | 31.73 | Room 24.9 C. |
| 12.40 | 31.435 | | 1.11 | 31.73 | |
| 12.41 | 31,445 | The warm water is now 40 C. | 1.12 | 31.735 | The warm water is now at 40.3 C. |
| 12.42 | 31.46 | At 12.42 right hand (not wrist) put into water at 8 C. Does not feel the cold at all. | 1.13 | 31.74 | At 1.13 dried and wrapped up right hand. |
| 12.43 | 31.485 | | 1.14 | 31.75 | |
| 12.44 | 31.50 | | 1.15 | 31.755 | |
| 12.45 | 31.515 | Room 24.8 C. | 1.16 | 31.765 | Room 24.9 C. |
| 12.46 | 31.525 | | 1.17 | 31.775 | |
| 12.48 | 31.54 | | 1.18 | 31.79 | |
| | | | 1.19 | 31.815 | |
| | | | 1.20 | 31.825 | Room 24.9 C. |
| | | | 1.21 | 31.835 | At 1.21 hand removed from calorimeter. Temperature of R. is 30.42 C. at 1.29. |
| | | | 1.29 | 31.76 | |

Cooling of R. 0.64° in 72 minutes; L. 0.08° in 8 minutes. Volume of right hand, 391 c.c.; left hand, 409 c.c.

Abdomen showed nothing abnormal. Liver and spleen did not seem to be enlarged. Genitalia were normal.

Reflexes: Knee-jerks, Achilles-jerks, Babinski, Gordon and Oppenheim reflexes all absent on both sides. Marked wasting of about the same muscles that were atrophied at the time of examination made in 1908, and described above; although the atrophy did not seem to be as pronounced and the strength in the hands was much better.

*Special Sense.*—He could taste neither sweet, sour nor bitter on the anterior two-thirds of the tongue on both sides. On the posterior one-third of the tongue he seemed to be able, especially on the left side, to taste acid, but no sweet or bitter substances. Sense of smell was absent. Hearing and sight were normal.

*Sensation.*—There was loss to all forms of sensation (including sense of position, deep pressure and vibratory sense) in the right hand extending up on the forearm with a U-shaped boundary on the anterior surface and extending much higher on the posterior surface, but here also having an irregular boundary outline (Figs. 1 and 2). Just above this area of complete anesthesia to all forms of sensation there was an area extending almost to the elbow joint where all forms of sensation were present, but less acute than normal. The left hand with the exception of the entire index finger was anesthetic to all forms of sensation. (At the blood-flow examinations he stated positively that he felt heat and cold only on the middle phalanx of index finger.) The upper boundary of anesthesia of the hand was about as high as the so-called glove anesthesia. Above this area was a band about 5 inches wide where all forms of sensation were present, but less acute than normal. Above this band there was another area extending almost to the elbow joint where all forms of sensation were again absent.

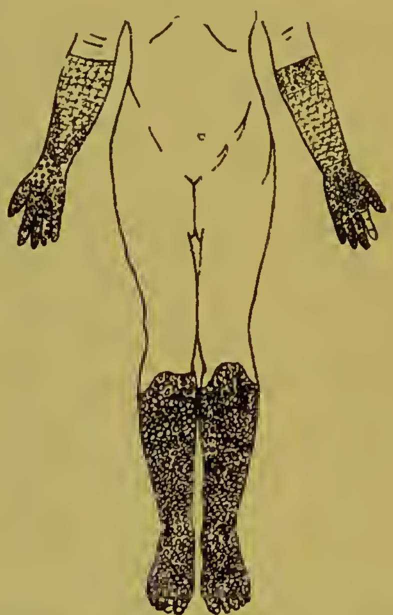

Fig. 1.—Anterior surface. Total anesthesia indicated by shading with small circles; hypesthesia (to all forms of sensation) by shading with small crosses.

The right foot and leg were anesthetic to all forms of sensation up to an irregular shaped line with its highest point three fingers' breadth below the patella. Posteriorly this line curves downward to a point six finger breadths below the level of the head of the fibula. Over this area all forms of sensation were lost, including muscle sense, deep pressure sense and vibratory sense. The left foot and leg showed almost identically the same area of anesthesia, with practically the same boundary, with the exception that there was a small round area about an inch in diameter on the sole of the foot just below the inner malleolus where pain sense seems to be present.

There was a sore on the plantar surface of the right big toe that seemed to be painful to touch. Patient thought it was caused by badly fitting shoes. It soon healed up perfectly. Numerous other abrasions due to shoes were not felt by the patient at all.

Marked displacement, laterally and in other direction, of the knees, ankles and toe joints was not perceived by the patient, but he seemed to appreciate the position of the left big toe.

It seemed as though vibratory sense was lost over the patella, fibula, tibia and bones of the feet. There seemed to be a lessening of the vibratory sense over the lower third of both thighs.

*Blood Examination.*—Blood examination showed 5,200,000 reds, 6,900 leukocytes and a negative Wassermann. Lumbar puncture showed rather low pressure (80 drops to the minute) clear, cell-count 4 per c.mm.

*X*-ray examination of the chest and spine showed nothing abnormal.

*Urine.*—Specific gravity 1,018, trace of albumin; no sugar and no casts found.

*Electrical Reaction.*—Electrical reaction showed greater diminution in the faradic than in the galvanic irritability in the areas affected by atrophy and anesthesia. Tested more exactly November 14, 1912, with a McIntosh battery (galvanic current from 25 cells with total voltage of 37. Faradic current from coil on the same apparatus actuated by two similar cells, No. 4 Columbia type 1½x4 inches in size connected in parallel). The patient stated that he could feel somewhat better on the hands and arms than at last examination, including heat and cold. Following are details of the electrical reactions:

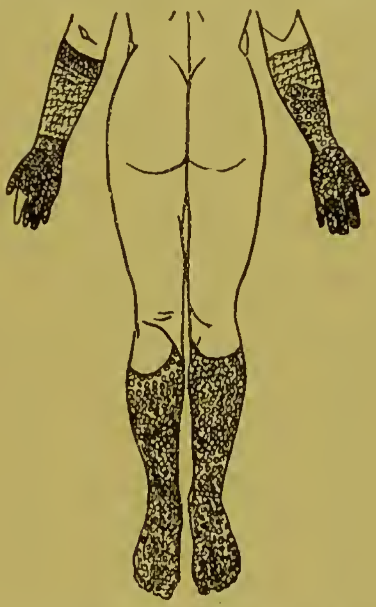

Fig. 2.—Posterior surface. Shading same as in Fig. 1. Note the small circular area on the left sole which seems to be sensitive to pain.

RIGHT ARM. GALVANIC CURRENT (BROAD ELECTRODE ON BACK[3]

N. Medianus (7 cm. above wrist): KCC>ACC. No KOC. Little if any AOC.

M. Palmaris Longus: KCC fair. ACC slight. No KOC or AOC. (Fairly sharp pain at KC and some pain at AC.)

M. Supinator Longus (Palm of hand): KCC (good) >ACC, which >AOC. KCC>KOC. No contraction either with K or A at C or O. (Slight pricking pain at AC, AO and KC. None at KO.)

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[3] The "motor points" correspond to those given by Schatzsky (Allbutt's System of Medicine, i, 454). It is not implied that the position of the points was identical in our patient, nor that only the corresponding muscle or nerve was always stimulated by the current used.

TABLE 5.—SUMMARY OF RESULTS ON R. E.

| Date | Temperature C. of | | | | Vol. of Hand in c.c. | | Heat Given Off in Small Calories | | | Blood Flow Gms. per Min. | | Flow per 100 c.c. of Hand per Min. | | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Room | R. Cal. | L. Cal. | Art. Bld. | R. | L. | R. | L. | In mins. | R. | L. | R. | L. | |
| April 26 | 24.0 | 30.82 | 30.69 | 36.6 | 379 | 397 | 951 | 1,469 | 15 | 12.19 | 18.41 | 3.21 | 4.56 | For the 15 mins. preceding the vasomotor test. |
| | .... | 30.85 | 30.78 | ..... | ... | ... | 459 | 598 | 6 | 14.78 | 19.02 | 3.90 | 4.71 | For 6 mins. immediately preceding the vasomotor test. |
| | 24.0 | ..... | 30.88 | ..... | ... | ... | ..... | 444 | 6 | ..... | 14.37 | .... | 3.56 | First 6 mins. immersion of right hand and wrist in warm water. |
| | .... | ..... | 31.04 | ..... | ... | ... | ..... | 1,161 | 8 | ..... | 29.00 | .... | 7.19 | Next 8 minutes. |
| | 23.6 | ..... | 31.18 | ..... | ... | ... | ..... | 287 | 3 | ..... | 19.61 | .... | 4.86 | First 3 mins. with right hand and wrist in cold water. |
| | .... | ..... | 31.22 | ..... | ... | ... | ..... | 636 | 12 | ..... | 10.94 | .... | 2.71 | Next 12 minutes. |
| May 2 | 25.4 | 31.10 | 31.19 | 36.6 | 361 | 385 | 541 | 578 | 10 | 10.93 | 11.87 | 3.03 | 3.08 | For 10 mins. before vasomotor test. |
| | 25.4 | 31.14 | ..... | ..... | ... | ... | 237 | ..... | 5 | 9.64 | ..... | 2.67 | .... | First 5 mins. immersion of left hand in warm water. |
| | 24.9 | 31.28 | ..... | ..... | ... | ... | 1,150 | ..... | 8 | 30.02 | ..... | 8.31 | .... | Next 8 minutes. |
| | 25.0 | 31.44 | ..... | ..... | ... | ... | 271 | ..... | 2 | 29.18 | ..... | 8.08 | .... | First 2 mins. with left hand in cold water. |
| | .... | 31.55 | ..... | ..... | ... | ... | 423 | ..... | 6 | 15.51 | ..... | 4.29 | .... | Next 6 minutes. |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| May 8 | 22.9 | 31.04 | 31.06 | 36.95 | 403 | 421 | 1,213 | 1,407 | 12 | 19.00 | 22.12 | 4.71 | 5.25 | For the 12 mins. preceding vasomotor test. |
| | 22.9 | ..... | 31.27 | ..... | ... | ... | ..... | 867 | 10 | ..... | 16.96 | .... | 4.03 | First 10 mins. with only anesthetic part of right hand in warm water. |
| | 22.8 | ..... | 31.41 | ..... | ... | ... | ..... | 1,012 | 10 | ..... | 20.29 | .... | 4.82 | Next 10 minutes. |
| | 22.8 | ..... | 31.52 | ..... | ... | ... | ..... | 329 | 4 | ..... | 16.83 | .... | 4.00 | First 4 mins. immersion of right wrist also in warm water. |
| | 22.8 | ..... | 31.63 | ..... | ... | ... | ..... | 1,009 | 8 | ..... | 26.33 | .... | 6.25 | Next 8 minutes. |
| | 22.7 | ..... | 31.78 | ..... | ... | ... | ..... | 686 | 8 | ..... | 18.42 | .... | 4.37 | First 8 mins. immersion of anesthetic part only of right hand in cold water. |
| | 22.7 | ..... | 31.83 | ..... | ... | ... | ..... | 282 | 5 | ..... | 12.24 | .... | 2.90 | Next 5 minutes. |
| | 22.8 | ..... | 31.85 | ..... | ... | ... | ..... | 460 | 9 | ..... | 11.13 | .... | 2.64 | For 9 mins. immersion of right hand and wrist in warm water. |
| May 18 | 24.8 | 30.98 | 30.97 | 36.55 | 391 | 409 | 1,158 | 1,317 | 18 | 12.83 | 14.57 | 3.28 | 3.56 | For 18 mins. preceding the vasomotor tests. |
| | 24.8 | 31.02 | 31.02 | ..... | ... | ... | 630 | 719 | 10 | 12.66 | 14.44 | 3.24 | 3.53 | For the last 10 mins. of this period. |
| | 24.6 | ..... | 31.11 | ..... | ... | ... | ..... | 383 | 6 | ..... | 13.04 | .... | 3.19 | First 6 mins. immersion of anesthetic part of right hand in cold water. |
| | 24.6 | ..... | 31.19 | ..... | ... | ... | ..... | 524 | 6 | ..... | 18.10 | .... | 4.42 | Next 6 minutes. |
| | 24.9 | ..... | 31.35 | ..... | ... | ... | ..... | 1,146 | 13 | ..... | 18.83 | .... | 4.60 | For 13 mins. during immersion of anesthetic part of right hand in warm water. |
| | 24.8 | ..... | 31.52 | ..... | ... | ... | ..... | 650 | 8 | ..... | 17.94 | .... | 4.38 | For 8 mins. immersion of anesthetic part of right hand in cold water. |
| | 24.7 | ..... | 31.61 | ..... | ... | ... | ..... | 616 | 12 | ..... | 11.54 | .... | 2.82 | For 12 mins. immersion right hand and wrist in cold water. |
| | 24.9 | ..... | 31.67 | ..... | ... | ... | ..... | 410 | 5 | ..... | 18.67 | .... | 4.56 | For the first 5 mins. immersion of right hand and wrist in warm water. |
| | 24.9 | ..... | 31.73 | ..... | ... | ... | ..... | 308 | 6 | ..... | 11.83 | .... | 2.89 | For the next 6 mins. of immersion in the warm water. |
| | 24.9 | ..... | 31.79 | ..... | ... | ... | ..... | 598 | 8 | ..... | 17.44 | .... | 4.26 | For 8 mins. with right hand dried and wrapped up. |

### LEFT ARM. GALVANIC CURRENT (BROAD ELECTRODE ON BACK)

N. Medianus (7 cm. above wrist): KCC (flexion of three middle fingers) >KOC. (Feels pain distinctly.) ACC (slight flexion of two middle fingers) is slightly less than KCC. AOC slight or absent. (Pain greater at AC than at AO.)

M. Palmaris Longus: No contraction, and he only just feels it.

M. Supinator Longus: KCC (fair) >KOC. ACC about the same as KCC. AOC is nearly as strong as ACC and stronger than KOC. Almost the strongest current yielded by the battery is needed to obtain even KCC.

M. Biceps: KCC (good) >KOC. Contractions easily obtained with rheostat 2 to 3 divisions from the maximum. The contractions are better than those elicited with the active electrode over the point "N. medianus" (7 cm. above wrist) immediately after, the current intensity indicated on the milliamperemeter being practically the same.

M. Extensor Carpi Ulnaris, M. Extensor Digiti Minimi, M. Extensor Indicis, M. Extensor Digitorum Communis: Fair contraction, both KCC and ACC, with rheostat three divisions from the maximum. (Pain felt rather strongly.)

N. Medianus (above elbow): KCC. Good contraction (flexion of fingers) and considerable pain.

The flexion of the fingers is greater than KCC with N. medianus (7 cm. above the wrist), but there is much less difference between the effects of the galvanic current on the two points than between the effects of the faradic current. With the galvanic current pain is quite distinct at both points although stronger at the point above the elbow.

Palm of Hand: KCC, fair movement of fingers, KCO very slight. (Only dull pains at KC.) ACO, fair contraction and >ACC.

### LEFT ARM. FARADIC CURRENT (BROAD ELECTRODE ON BACK)

N. Medianus (7 cm. above wrist): No contraction and very little sensation with shield one-third withdrawn. Some contraction with core completely uncovered by withdrawing shield entirely.

N. Medianus (above elbow): Good contraction (flexion of fingers) with core one-third uncovered and pain. Much stronger contraction with shield completely withdrawn than at the lower N. medianus point.

M. Palmaris Longus: Fair contraction with shield one-third withdrawn. The contraction is mainly proximal to the point.

M. Supinator Longus: Fair contraction with shield half withdrawn.

M. Biceps: Good contraction with weakest current (shield not withdrawn at all). He feels it well.

M. Extensor Carpi Ulnaris, M. Extensor Digiti Minimi, M. Extensor Indicis, M. Extensor Digitorum Communis: Contraction with shield one-fourth withdrawn. The contraction is mainly proximal to the points stimulated.

### RIGHT ARM. FARADIC CURRENT (ONE ELECTRODE IN PALM)

N. Medianus (7 cm. above wrist): No contraction whatever with maximum strength. He feels some pain but not much.

M. Supinator Longus: With maximum strength some contraction (dorsiflexion of thumb, index and middle fingers). Pricking sensation mostly in the palm; only a little at position of the other electrode.

### LEFT ARM. FARADIC CURRENT (ONE ELECTRODE IN PALM)

N. Medianus (7 cm. above wrist): With maximum strength some contraction of muscles of ball of thumb, etc.

M. Supinator Longus: Fair contraction with maximum strength.

M. Palmaris Longus: No contraction except with maximum strength, which gives fair flexion to fingers.

Four blood-flow experiments on as many separate days during a period of three weeks were made on the hands of this patient and one on his feet. The experiments on the feet will be reserved for inclusion in a separate paper on the blood-flow in the feet. A fifth experiment on the hands was partially vitiated for its immediate purpose by the fact that the man came to the laboratory on a rather cold morning with his hands uncovered. This caused a persistently diminished flow in the hands, as is particularly apt to be the case in persons with a habitually poor flow, so that the existence of reflex vasomotor effects from one hand to the other could not be studied under favorable conditions. Nevertheless, the results in this experiment, although quantitatively less marked, were qualitatively the same as in the others.

The question whether in this patient the efferent vasomotor path in the areas where sensation is entirely gone is still capable of conducting vasomotor impulses was studied in the experiments of April 26 and May 2, in which the vasomotor reflexes elicited in one hand by the application of heat or cold to sensitive areas on or near the other hand were determined. The reflex effects on the blood-flow were normal in direction and of considerable intensity.

Thus, in the experiment of April 26 the flow for a period of six minutes before the vasomotor reaction was tested, was 3.90 grams per 100 c.c. of hand per minute in the right hand, and 4.71 grams in the left. For a fifteen-minute period, immediately preceding the vasomotor test, the flows were, respectively, 3.21 and 4.56 grams. The flow in both hands was slowly increasing up to six or eight minutes before the test. The right hand, it will be remembered, is the weaker of the two, a condition which, in old-standing neuritis, has been found associated with a smaller flow. In neither hand is the flow of normal amount for the age of the patient, and the temperature of the room and calorimeters. This agrees entirely with the fact that his hands were always cold to the touch, although he himself did not know that they were cold. When the right hand was immersed in warm water so deeply that warmth was distinctly felt on the wrist, the flow in the left hand for the first six minutes of the immersion was reduced to 3.56 grams per 100 c.c. of hand. A rather abrupt increase then occurred, the average flow for a further period of immersion of eight minutes in the warm water being 7.19 grams per 100 c.c. of hand per minute. The initial diminution of the flow in the hand when the contralateral hand is immersed in warm water is the normal effect, only in this case the diminution is more prolonged than normal. On immersion of the right hand and wrist in cold water, which was well felt on the wrist, the flow in the left was diminished from the average of 7.19 grams per minute to 4.86 grams for the first three minutes and to 2.71 grams for the next twelve minutes, while the right hand continued in the cold water. The vasoconstriction and vasodilatation in the left hand elicited by thermal stimuli applied to the right hand and appreciated only on the right wrist were therefore perfectly distinct.

In the experiment of May 2 the initial flows were 3.03 and 3.08 grams per 100 c.c. per minute for the right and left hands, respectively. When the left hand was immersed in warm water so that the sensation of warmth was only appreciated on the middle phalanx of the left index finger, although there quite intensely, a vasomotor reflex occurred in the contralateral hand with exactly the same features as in the experiment of April 26, viz., a longer initial period than usual, in which the flow was diminished by the application of warmth to the contralateral hand (from 3.03 to 2.67 grams per 100 c.c. per minute for a period of five minutes) succeeded by a very durable increase (to 8.31 grams per 100 c.c. per minute) for the remaining eight minutes, during which the left hand continued in the warm water. The reaction to the immersion of the left hand in cold water was also quite distinct (an eventual reduction of flow in the right hand from 8.31 to 4.29 grams per 100 c.c. per minute).

It is noteworthy, and of course, quite in accord with experimental facts, that a wide-spread and intense vasomotor reflex may be initiated from a very limited receptive area. Therefore, the intensity of the vasomotor reflex in those two experiments is no indication that any portion of the reflex has been elicited from the anesthetic areas of the hands immersed in the warm or cold water. We shall see, however, later on that even the anesthetic areas do contribute something to the total effect, although the contribution is relatively slight.

From these experiments it can be concluded (1) that the efferent vasomotor paths in the parts which are totally anesthetic in this patient are quite capable of conduction; (2) that the centers concerned in reflex vasomotor action on the blood-vessels of a totally anesthetic hand and elicited by heat and cold from areas on or near the anesthetic contralateral hand are quite permeable for vasomotor impulses.

The vasomotor reflex is so good that we can assume that practically the whole efferent vasomotor path to the hand is concerned in it. Therefore, when it is elicited from so limited a receptive surface the relatively small number of fibers in the afferent path must have free access either directly or through the bulbar vasomotor centers to the spinal cells of origin of the afferent fibers for practically the whole of the contralateral hand, not to speak of other areas which very probably are involved in the reaction. This could not well be the case if a lesion existed in the cord, and in the immediate vicinity of the cells of reception of the small afferent path, extensive enough to account for the sensory and motor deficiency in the hands. Assuming, then, that of the three links in the vasomotor reflex arc, two (the efferent path and the central mechanism) are absolutely or relatively intact, we have now the means of determining the condition of the third link (the peripheral afferent path). This was done in the experiments of May 8 and May 18, in which the behavior of the reflexes in one hand when only the anesthetic parts of the contralateral hand were exposed to heat or cold were studied. It is there shown that the reflexes elicited from the anesthetic areas are feeble in comparison with those elicited from adjacent areas in which sensation is preserved. Thus, in the experiment of May 8 the initial flow in the left hand was 5.25 grams, in the right hand 4.71 grams per 100 c.c. per minute. For the first ten minutes of immersion of the anesthetic portions of the right hand in warm water the flow in the left hand was 4.03 grams per 100 c.c. per minute, which increased to 4.82 grams during continued immersion of the right hand (still only the anesthetic portions) for an additional period of ten minutes. Great care was taken that no part of the hand should be immersed in which the patient preserved any power whatever of temperature sensation. The wrist was carefully protected by cloths from contact with the vessel. When the wrist of the right hand as well as the anesthetic portions of the hand were immersed in warm water so that the patient experienced a distinct sensation of warmth on the wrist, the flow in the left hand, after an initial diminution from 4.82 grams to 4.0 grams per 100 c.c. per minute for a period of four minutes (the normal effect), increased to 6.25 grams during continued immersion of the hand and wrist in the warm water for an additional period of eight minutes. Immersion of the anesthetic part of the right hand in cold water caused the flow in the left hand to diminish to 4.37 grams for the first eight minutes of immersion. The rest of the experiment probably should not be used, as the marked diminution in the flow is a phenomenon often witnessed at the end of long experiments.

In the experiment of May 18 the initial flows were 3.28 and 3.56, respectively, for the right and left hand. Immersion of the right hand (only the anesthetic portions) in cold water caused the flow in the left hand to diminish from 3.56 to 3.19 grams for the first six minutes of immersion. During the next six minutes of immersion of the right hand in cold water the flow increased to 4.42 grams per 100 c.c. per minute, the increase, as is usually the case even in normal hands, beginning abruptly. On immersion of the anesthetic portions of the right hand in warm water a further small increase (to 4.60 grams per 100 c.c. per minute) occurred and this was sustained during the total period of immersion of the right hand in warm water thirteen minutes. Immersion of the anesthetic portions of the right hand in cold water now caused a slight diminution of the flow in the left hand (from 4.6 to 4.38 grams per 100 c.c. per minute for a period of eight minutes). Changes of a very different order of magnitude were produced when the right hand including the wrist was now immersed in cold water, the flow in the left hand falling from 4.38 to 2.82 grams per 100 c.c. per minute for the twelve minutes of immersion. The patient felt the cold intensely on the right wrist, not at all on the rest of the hand. Immersion of the right hand and wrist in warm water caused an increase in the flow in the left hand from 2.82 grams to 4.56 grams per 100 c.c. per minute for a period of five minutes. During the next six minutes, while the right hand and wrist were still immersed in the warm water, the average flow in the left hand sank to 2.89 grams (probably vasoconstriction due to the great duration of the experiment). When the right hand was removed from the warm water, dried carefully and wrapped up in a cloth, the flow in the left hand rose again to 4.26 grams per 100 c.c. per minute.

## DISCUSSION AND CONCLUSIONS

From these four experiments taken together the following conclusion seems justified: Since there is no conspicuous defect of conductivity either in the centers or in the efferent vasomotor paths as shown in the first two experiments, and incidentally also in the last two, the defect in the experiments in which only anesthetic areas were exposed to warmth or cold must lie in the afferent portion of the arc; that is to say, in the afferent path from the anesthetic regions through which vasomotor reactions in the contralateral hand can be normally elicited. Further, the lesion as regards the vasomotor path is in all probability a peripheral one involving mainly the afferent fibers and at a level at any rate distal to their cells of reception in the spinal gray matter. The suggestion is that the lesion in the other afferent paths is also peripheral, a question of diagnosis left in doubt by previous clinical examinations. This supports the diagnosis of the case as post-typhoidal neuritis of unusually long duration and with other unusual features.

Perhaps, roughly speaking, we might arrange the various paths as regards the severity of the peripheral lesion as follows: 1. Sensory, including tactile and thermal sensation, pain and also deep sensibility; for example, vibratory. 2. Afferent vasomotor. 3. Voluntary motor. 4. Efferent vasomotor. It is not implied that the whole change is necessarily in the peripheral fibers nor is it perhaps probable, but only that the peripheral fibers are mainly affected. The classification is purely functional. It is not implied, for instance, that Group 2 is anatomically distinct from Group 1. There is, of course, no inherent improbability in the supposition that the efferent and afferent portions of the vasomotor arc are differently affected. The distal neurons of the two paths belong to different anatomical systems, the efferent to the autonomic, the afferent to the cerebrospinal system.

While the changes in the flow in one hand produced by immersing the completely anesthetic portion of the contralateral hand in cold or warm water are not large in comparison with the changes elicited by the application of heat or cold to parts with good temperature sensibility, they seem sufficiently definite to permit the conclusion that even from a part completely anesthetic as regards temperature sensation (as well as regards the other forms of sensation) vasomotor reflexes can be evoked by cold or warmth. Two explanations might be thought of:

1. The block which conditions the anesthesia is partially central, i. e., somewhere above the entrance of the afferent fibers into the cord. In this case the afferent sensory paths from the anesthetic areas need not be looked on as totally incapable of conduction of sensory impulses and therefore not incapable of conducting impulses which can cause reflex vasomotor effects.

2. The peripheral block being complete for sensory impulses, it is still incomplete for afferent vasomotor impulses. The second condition might depend (a) on the possibility of the reception and conduction by the nerves of skin sensation (particularly in the case we are discussing the nerves which subserve the sensations of warmth and cold) of impulses which can affect reflex vasomotor centers even when they are incapable of receiving and conducting impulses which give rise to the sensations in question, or at least incapable of receiving and conducting them in sufficient intensity to reach threshold value; (b) on the possibility that other afferent fibers than those concerned in sensation exist in the peripheral nerves, through which impulses elicited by heat or cold may pass to vasomotor centers. If such fibers exist there is no reason why they should be involved in exactly the same degrees as the sensory fibers in a chronic peripheral pathological process which is nothing if not selective.

That it is not necessary to make the assumption involved in explanation 1 was shown by experiments on a surgical case in which a central lesion could be positively excluded. By an injury to the median nerve in the forearm, certain phalanges were rendered totally anesthetic to warmth, cold and the other forms of cutaneous sensation. A small, but distinct, reflex vasomotor effect on the contralateral hand was obtained on immersion of the anesthetic phalanges in warm or cold water. The subject, a medical student, thoroughly appreciated the object of the experiment, and his statement that during the immersion of the anesthetic phalanges no temperature sensation whatever was experienced, can be taken as strictly accurate. In this case then, the afferent vasomotor path from the anesthetic phalanges was already in some degree permeable when excited by heat or cold at a time when no thermal sensation had returned. As the case had not come under observation at an earlier date it is impossible to say whether the degree of conductivity observed was a residual phenomenon due to the sparing of the path, or of a portion of it, in the original lesion, or to recovery. It is, of course, conceivable that impulses which can liberate vasomotor reflexes may be capable of being received by, and of passing up, regenerating nerve fibers at a much earlier period than impulses which give rise to sensations. This case is being further studied as regards the return of function by the subject himself under the direction of one of us and will form the basis of a separate paper. The question whether thermal vasomotor reflexes can be elicited from deeper structures than the skin, from muscles, for example, whose afferent fibers when electrically stimulated cause such marked vasomotor effects, has an obvious relation to this subject. We have not discussed it because we do not possess the data for doing so. In any case it is certain that the skin, which must bear the brunt of those changes in the external temperature, the adaptation to which is one of the great offices of the vasomotor mechanism, must be far more important in this regard. We have also made no allusion to the possibility that temperature changes, due either to direct conduction or to the passage of the blood from the submerged parts to neighboring parts not involved, or not involved in an equal degree in the lesion, may affect afferent nerves capable of liberating vasomotor reflexes, because if such changes occurred they were too slight or too gradual to elicit sensations of cold or warmth, and it was only with temperature changes which did this that we concerned ourselves.

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# VASOMOTOR REFLEXES ELICITED BY HEAT AND COLD FROM AREAS DEPRIVED OF TEMPERATURE SENSIBILITY BY A TRAUMATIC LESION *

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In a pathological case already reported in this journal[1] it was shown that vasomotor reflexes, small but definite, could be elicited by the application of heat or cold to areas totally devoid of temperature sensibility. The present communication establishes the same fact, for an otherwise normal subject, in whom anesthesia of certain portions of one hand had been caused by accidental injury to the median nerve in the fore-arm.

## CASE REPORT

*History.*—The subject, O. C. W., is a healthy fourth-year medical student, aged 24. September 15, 1912, at 9:30 a. m., he injured his left arm by the breaking of a carboy full of water which he was carrying. The main wound was on the front of the lower part of the forearm. In addition there was a small wound on the dorsal aspect of the little finger. From the way in which the carboy was being held the tendons in the lower part of the forearm and at the wrist were on the stretch and stood out, so that they could easily be severed by a relatively superficial wound. The bleeding, so far as could be observed, was entirely venous and capillary. Certainly no large artery was cut. The hemorrhage was controlled by the patient himself by pressure around the wrist with the right hand until assistance was procured. He was taken to a hospital and under gas anesthesia eight flexor tendons which were found divided were sutured, and in addition the tendon of the extensor digiti quinti propius on the dorsum of the little finger. A careful search was made in the wound for divided nerves but no large nerve was found. The wound was closed and a splint put on the arm and hand. The arm was dressed morning and evening for the first few days and afterward each day, and healing occurred without complication.

During the interval (4½ hours) between the injury and the operation the patient was able to make the following observations.

*Motor Changes.*—Immediately after the accident it was impossible to flex either the thumb or the index finger or any of their phalanges, but adduction and abduction of the thumb and index were not impaired. The middle, ring and little fingers could be flexed, the deep tendons being intact. The hand could not be flexed on the wrist. The little finger could not be extended.

*Sensory Changes.*—The entire index finger became numb immediately. This numbness later involved more or less the whole of the hand, but was most marked on the radial side of the palmar aspect.

The patient continued from time to time to make observations.

September 20, 1912. The numbness, except on the index finger, has changed into an intense burning and tingling sensation which involves the thumb, middle finger, radial side of ring finger and the radial half of the palm. The little finger

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[1] Stewart and Laffer: The Archives Int. Med., 1913, xi, 365.

and the ulnar half of the palm are not involved in this sensation and the same is true of the dorsal, ulnar and palmar surfaces of the ring finger. The skin over the area involved in this sensation as well as that over the index finger is very dry. The furrows of the skin in this region are noticeably white. There is some swelling in the index and middle fingers.

September 30, 1912. The splint was removed to-day. The sensation of burning and tingling remains the same and is limited as above described. It is like the pain of a burn exposed to the air and is worse at night. The skin has the same appearance as before and is still very dry. On the palmar surface of the third phalanx of the middle finger a small area about 1 cm. in diameter can now be delimited which is very sensitive to light stroking (cotton wool or brush). This hypersensitive spot was first noticed in the latter part of the first week. This area and the rest of the phalanx are anesthetic as regards pain (pin prick), deep pressure, heat and cold. The anesthetic area (to touch, pain, pressure, heat and cold) involves the palmar surface of the thumb, the radial half of the palm, the palmar surface of the index and middle fingers and the entire periphery of the second and third phalanges of the index and middle fingers. The radial side of the ring finger has a certain degree of hypesthesia. The involved area is more or less sensitive to light stroking, the middle finger most, the index finger least. On the middle finger especially such stroking is quite painful while hard rubbing causes no pain. A mere touch on the middle finger, especially the palmar surface, caused discomfort, whereas prolonged stroking or (light) rubbing was necessary to produce the same sensation on the index finger. This hypersensitive condition appeared about the second week while the splint was still on. Sensation is normal on the dorsum of the thumb up to and around the nail, also on the dorsum of the first phalanx of the index and middle fingers. The sides of the first phalanx of the index and middle fingers are normal. The skin over the involved area is desquamating. Motion of the whole hand and of the fingers is greatly limited.

October 10, 1912. Motion much improved. Sensation the same as before, but the painful sensations are less intense. The palm and palmar surface of the middle finger are not so sensitive to light contact (stroking). Cannot localize contact. Cannot distinguish pressure at two points. Thermic sensation absent in the entire involved area.

October 20, 1912. Motion better. Can flex all fingers into the palm. Deep flexor tendon to index does not work, does not flex the terminal phalanx. Tactile sensibility is not appreciably improved. Localization and discrimination of the shape, etc., of a body touching the skin are absent.

November 10, 1912. Motion better. Otherwise no change.

December 10, 1912. Skin over the involved area not so dry. Obviously there is now some sweat secretion but still much less than on the normal skin. Occasional shooting pains starting about the wound and shooting to the ends of the index and middle fingers. On tapping lightly over the scar a sensation similar to that caused by a weak electric shock is transmitted to the ends of the index and middle fingers, never transmitted elsewhere.

December 19, 1912. Accidentally burnt the palmar surface of the terminal phalanx of the index finger. The finger was in contact with a radiator and the patient was quite unconscious it was being burnt. A large blister formed in about an hour.

December 20, 1912. The blister was opened and drained by puncturing with a needle through healthy skin at the side. It began to fill immediately and in a few minutes was as large as before. It was reopened and filled again very quickly and this process was repeated about fifteen times during the day.

December 21, 1912. The skin over the blister was cut off and the accumulation of liquid stopped at once. There was no recurrence of the formation of liquid. No pain was felt from the burn at any time. It was so deep as to involve the fat pad. By January 7, 1913, the burn was completely healed.

December 23, 1912. The distribution of sweat secretion and sensation was carefully tested in the laboratory.

*Sweat Secretion.*—There is no question that the area deficient in sweat secretion is much smaller than at first, or even than a month ago. The hand was wrapped in a towel after being wiped dry and put into a hot-air oven. The oven contained a dish of water to help to keep the air moist and thus hinder evaporation of the sweat. Sweat secretion is still small or absent over an area on the ball of the thumb about 3½ cm. long by 2 cm. broad. The edge of this area approaches the cleft between the thumb and index finger. On the rest of the radial portion of the palm and on the second and third phalanges of the middle finger, where sweating was absent before, it is now present to a certain extent.

*Sensation, Radial Half of Palm.*—The small area on the ball of the thumb in which sweat secretion is absent is totally insensitive to light touch (with blunt pencil point, cotton wool or brush). To pressure it is sensitive. If a blunt point is pressed down with more than a certain amount of force the pressure sensation goes on to definite though delayed pain sensation. This area is less sensitive to pressure than the rest of the ball of the thumb. With a tube containing water at 3 C. no cold sensation on this small area, but with a chip of ice a sensation of cold is obtained though delayed. No sensation of warmth with a tube containing water at 45 C., nor was there any effect for some distance outside the boundary. Hotter water (50 C.) is felt as warm on points just outside although not felt at all inside the boundary, whereas elsewhere on the palm it is felt as hot. The rest of the radial half of the palm is sensitive to touch (light contact of blunt point, light stroking with cotton or brush), pressure (with rubber pad of pencil), pain (pin-prick), heat and cold (chip of ice). While ice causes cold sensation everywhere on the palm of the hand it is somewhat less marked on the ball of the thumb and over the radial half of the palm than over the rest of the palm, rather a cool sensation than that of ice.

*The thumb* is sensitive to light touch but on the palmar surface of the second phalanx, although a tactile sensation is elicited, the subject cannot tell anything about the kind of body touching him; for example, whether it is pointed or not. Pressure sensation is fair on the palmar surface of the thumb and good on the dorsum. Ice gives no sensation of cold on the palmar surface of the second phalanx; elsewhere the normal sensation. The pin-prick, which elsewhere on the thumb causes pain, elicits only the sensation of contact on the palmar surface of the second phalanx.

*The little finger* is normal. The more detailed examination of the remaining three fingers follows.

*Light Touch.—Index Finger, Palmar Surface.*—On the second and third phalanges no sensation to simple touch but some sensation to pressure, not going on to pain even with strong pressure with a blunt point. On the first phalanx touch is felt but the shape of the object is not discriminated. *Dorsal Surface.*—Third phalanx only slight sensation of touch without discrimination of nature of object. Second phalanx much better touch sensation. First phalanx normal. *Ulnar Surface.*—Third phalanx no tactile sensation. Second phalanx fair, third normal. *Radial surface.*—Third phalanx poor tactile sensation, second pretty good, first normal.

*Middle Finger.*—Hyperesthetic to light touch on palmar aspect, elsewhere much the same as the index.

Light stroking with cotton wool or brush shows much the same distribution of anesthesia over the whole involved area as in the other tests.

*Cold.—Index, Palmar Surface.*—Second and third phalanges no cold sensation to ice. First phalanx, little if any cold sensation, perhaps a slight sensation after a long application of the ice. *Dorsal and Radial Surfaces.*—Second and third phalanges no cold sensation. First phalanx normal. *Ulnar Surface.*—Second and third phalanges, no cold sensation. First phalanx, cold sensation but delayed.

*Middle Finger, Palmar Surface.*—No cold sensation on any of the phalanges, but the contact of ice gave a sensation of touch on all. *Dorsal, Radial and Ulnar Surfaces.*—Second and third phalanges, no cold sensation; first phalanx normal.

*Ring Finger.*—Palmar, dorsal and ulnar surfaces normal. *Radial Surface.*—First and third phalanges normal; a small area about 5 mm. distal to the joint between the first and second phalanges on the radial side and overlapping the dorsal surface of the second phalanx either gives no cold sensation with ice or the sensation is markedly delayed.

The application of a point of ice to the point on the scar in the forearm, mechanical stimulation (tapping) of which gives a sensation like a weak electric shock running down to the index and middle fingers causes no effect except a local sensation of cold over the point touched. On no part of the anesthetic area is anything except a local sensation of cold obtained on contact with ice. No "peripheral reference" of cold[2] could be demonstrated at this stage at any part of the anesthetic area.

*Warmth (warm tube).—Index Finger, Palmar Surface.*—No sensation of warmth on any phalanx. Sensation of contact on first phalanx. *Dorsal Surface.*—Warmth felt on first phalanx, nothing on second and third.

*Middle Finger.*—The same portions are sensitive to warmth as to cold and in about the same degree.

*Ring Finger.*—Normal except the small area on the second phalanx which was insensitive to ice. This area gives the sensation of warmth with a tube which elsewhere gives a hot sensation.

*Pain (pin-prick).—Index Finger, Palmar Surface.*—Only sensation of contact is given on first phalanx except with considerable pressure, when pain is elicited. Second and third phalanges give only sensation of contact to pin-prick and heavy pressure is required to elicit even this. No discrimination of the nature of the object. *Dorsal Surface.*—First phalanx normal. Second phalanx greater pressure of the pin needed to give pain than on normal skin. Third phalanx no pain, even with considerable pressure; only an indefinite contact sensation.

*Middle Finger, Palmar Surface.*—All the phalanges are hyperesthetic. A pin-prick gives definite pain, much more intense than on the rest of the skin. Second and third phalanges are more sensitive than the first. Light contact or a light prick gives most pain. Pressing or rubbing hard with a flat surface does not give pain, nor does pinching. Light stroking gives a sensation of exaggerated tickling which is almost pain.

*Ring Finger.*—Normal to pin-prick except the small area which is abnormal to temperature sensation. On this area a pin-prick gives sensation of contact and not of pain.

*Pressure (with rubber pad of pencil).—Index, Palmar Surface.*—First phalanx, fair sensation, diminishing on second phalanx toward third phalanx. *Dorsal Surface.*—First phalanx normal. On third phalanx pressure sensation is barely obtainable. Second phalanx intermediate.

*Middle Finger, Palmar Surface.*—Good pressure sensation on all phalanges but accompanied with tingling. *Dorsal Surface.*—First phalanx normal. Second phalanx fair sensation, and better than on second phalanx of index finger. Third phalanx about the same as second phalanx of index finger; on increasing the pressure the pressure sensation goes on to pain, which is not the case on the second and third phalanges of the index finger.

*Ring Finger.*—Normal everywhere.

January 21, 1913. Light touch (stroking with brush) is felt over the whole of the involved area but only slightly on the second and third phalanges of the index finger. On the palmar surface of the middle finger the sensation is one of exaggerated tickling. No area can now be found on the ball of the thumb which is insensitive to contact (brush or blunt pencil point). The radial portion of the palm is moist althrough dryer than the rest of the palm.

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[2] Trotter and Davies: Jour. Physiol., 1909, xxxviii, 178.

January 24, 1913. Peripheral reference of cold cannot be obtained. Sensation of cold over the scar is delayed.

The subject came under observation of the senior author for the first time, a month after receiving the injury. The first blood-flow examination was made Oct. 15, 1912.

October 15, 1912. The blood flow and vasomotor reflexes of the hands were examined after the subject's day's work as a medical student. Pulse 112 (sitting). Hands in bath at 4:12 p. m. Hands in calorimeters at 4:35 p. m. The hands were kept in the bath for a longer time than usual owing to accidental delay in the preparations: 3,015 c.c. of water in each calorimeter. Volume of right hand 447 c.c., of left 436 c.c. Rectal temperature 37.15 C. Height 5 feet, 7½ inches. Weight 146 pounds. Came in from street not wearing gloves.

TABLE 1.—Calorimetric Tests of Blood-Flow in Hands of O. C. W.

| Time. | R. | L. | Notes. |
|---|---|---|---|
| 4:34 | 31.02 | 31.10 | |
| 4:36 | 31.02 | 31.10 | |
| 4:37 | 31.04 | 31.12 | |
| 4:38 | 31.07 | 31.15 | Room 22.6 C. |
| 4:39 | 31.10 | 31.19 | |
| 4:40 | 31.13 | 31.22 | Room 22.6 C. |
| 4:41 | 31.20 | 31.26 | |
| 4:42 | 31.25 | 31.31 | Room 22.5 C. |
| 4:43 | 31.31 | 31.34 | |
| 4:44 | 31.38 | 31.39 | |
| 4:45 | 31.42 | 31.43 | Room 22.8 C. |
| 4:46 | 31.46 | 31.46 | |
| 4:47 | 31.495 | 31.495 | |
| 4:48 | 31.52 | 31.52 | |
| 4:49 | 31.535 | 31.53 | |
| 4:50 | 31.56 | 31.55 | Room 22.9 C. |
| 4:51 | 31.56 | 31.56 | |
| 4:52 | 31.585 | 31.57 | Room 22.9 C. |
| 4:53 | 31.595 | 31.585 | † * |

| Time. | R. | Notes. |
|---|---|---|
| 4:54 | 31.595 | Room 22.9 C. |
| 4:55 | 31.61 | |
| 4:56 | 31.65 | |
| 4:57 | 31.69 | Room 23.1 C. |
| 4:58 | 31.71 | |
| 4:59 | 31.73 | Room 23.1 C. |
| 5:00 | 31.785 | |
| 5:01 | 31.81 | Room 23.0 C. |
| 5:02 | 31.825 | At 5:02 anesthetic phalanges immersed in water at 43.4 C. |
| 5:03 | 31.845 | |
| 5:04 | 31.89 | |
| 5:05 | 31.91 | |
| 5:06 | 31.96 | |
| 5:07 | 32.01 | |
| 5:08 | 32.08 | |
| 5:09 | 32.11 | |
| 5:10 | 32.16 | At 5:10 whole left hand put into water at 42.6 C. |
| 5:11 | 32.18 | |
| 5:12 | 32.22 | Room 23.3 C. |
| 5.13 | 42,265 | |
| 5:14 | 32.29 | |
| 5:15 | 32.335 | |
| 5:16 | 32.395 | |
| 5:17 | 32.45 | |
| 5:18 | 32.48 | At 5:18 whole left hand put into cold water. |
| 5:19 | 32.495 | Room 23.4 C. |
| 5:20 | 32.505 | |
| 5:21 | 32.535 | Felt the cold water sharply at first but is now getting used to it. |
| 5:22 | 32.57 | |
| 5:23 | 32.59 | |
| 5:24 | 32.60 | Room 23.35 C. |
| 5:25 | 32.61 | |
| 5:26 | 32.625 | Hands out of calorimeter at 5:26. |
| 5:38 | 32.46 | Temp. of L. is now 31.04 C. |
| .... | ..... | Cooling of R. 0.165 degrees in 12 minutes. |
| .... | ..... | Cooling of L. 0.545 degrees in 45 minutes. |

† At 4:53 the anesthetic phalanges of left hand immersed in water at 8.2 C.

* The hands were cautiously immersed so that the subject should not feel the cold water, the hand and wrist being kept from contact with the vessel by a towel. On their first introduction into the cold water the anesthetic phalanges were immersed for a moment rather too deeply, so that he felt what he termed a suspicion of cold. The fingers were at once withdrawn a little and thereafter he had no sensation of cold whatever.

In this experiment immersion of the anesthetic phalanges of the left hand in cold water caused a definite though transient diminution of the flow in the right hand (from 7.39 to 3.82 grams per 100 c.c. per minute) for the first two minutes of immersion. For the next seven minutes while the anesthetic phalanges continued in the cold water the flow increased to 7.58 grams. For the whole nine minutes the flow was diminished from 7.39 to 6.75 grams per 100 c.c. of hand per minute. The initial flow in the left hand was somewhat less than that in the right (6.83 grams per 100 c.c. per minute). A considerable and more persistant increase in flow followed the immersion of the anesthetic phalanges in the warm water (from 7.58 to 10.58 grams per 100 c.c. of hand per minute for a period of eight minutes). When the whole left hand was now immersed in the warm water some further increase in the flow of the right hand (to 11.14 grams per 100 c.c. per minute for a period of eight minutes) ensued, and this was promptly cut down to 7.24 grams when the whole left hand was immersed in the cold water. Not only was the diminution greater when the whole hand was immersed, but it was

TABLE 2.—CALORIMETRIC TESTS OF BLOOD-FLOW IN HANDS OF O. C. W.

| Time. | R. | L. | Notes. |
|---|---|---|---|
| 2:11 | 30.93 | 30.90 | Room 23.1 C. |
| 2:13 | 30.89 | 30.93 | |
| 2:14 | 30.93 | 30.99 | Room 23. 3 C. |
| 2:15 | 30.99 | 31.04 | |
| 2:16 | 31.06 | 31.10 | Room 23.3 C. |
| 2:17 | 31.12 | 31.16 | |
| 2:18 | 31.195 | 31.205 | Room 23.3 C. |
| 2:19 | 31.265 | 31.265 | Room 23.3 C. |
| 2:20 | 31.31 | 31.31. | |
| 2:21 | 31.36 | 31.34 | Room 23.2 C. |
| 2:22 | 31.40 | 31.39 | Room 23.3 C. |
| 2:23 | 31.44 | 31.41 | At 2:23 anesthetic phalanges put into water at 8 C. |

| Time. | R. | Notes. |
|---|---|---|
| 2:24 | 31.46 | |
| 2:25 | 31.48 | Room 23.3 C, |
| 2:26 | 31.515 | |
| 2:27 | 31.55 | Room 23.3 C. |
| 2:28 | 31.59 | |
| 2:29 | 31.63 | Room 23.2 C. |
| 2:30 | 31.69 | Room 23.3 C. |
| 2:31 | 31.73 | |
| 2:32 | 31.78 | Room 23.3 C. |
| 2:33 | 31.805 | At 2:33 anesthetic phalanges put into water at 43.5 C. |
| 2:34 | 31.825 | |
| 2:35 | 31.85 | |
| 2:36 | 31.89 | |
| 2:37 | 31.92 | |
| 2:39 | 31.96 | Room 23.4 C. |
| 2:39 | 31.99 | |
| 2:40 | 32.04 | |
| 2:41 | 32.08 | Room 23.4 C. |
| 2:42 | 32.115 | |
| 2:43 | 32.16 | Room 23.3 C. |
| 2:44 | 32.195 | At 2:44 whole left hand put in water at 43 C. Room 23.3 C. |
| 2:45 | 32.32 | |
| 2:46 | 32.265 | |
| 2:47 | 32.30 | |
| 2:48 | 32.355 | Room 23.3 C. |
| 2:49 | 32.395 | |
| 2:50 | 32.44 | Room 23.3 C. |
| 2:51 | 32.51 | |
| 2:52 | 32.57 | Room 23.3 C. |
| 2:53 | 32.61 | |
| 2:54 | 32.67 | |
| 2:55 | 32.705 | At 2:55 whole left hand put in water at 8 C. |
| 2:56 | 32.74 | Room 23.1 C. |
| 2:57 | 32.775 | |
| 2:58 | 32.795 | |
| 2:59 | 32.825 | |
| 3:00 | 32.85 | |
| 3:01 | 32.875 | Room 23.3 C. |
| 3:02 | 32.895 | |
| 3:03 | 32.895 | |
| 3:04 | 32.90 | At 3:04 dried and wrapped left hand in warm cloth. |
| 3:05 | 32.91 | |
| 3:06 | 32.925 | Room 23.3 C. |
| 3:07 | 32.935 | Room 23.1 C. He says the left hand feels warm. |
| 3:08 | 32.955 | |
| 3:09 | 32.97 | |
| 3:10 | 32.975 | |
| 3:11 | 32.975 | Room 23.3 C. |
| 3:12 | 33.02 | Hand removed from calorimeter at 3:12. |
| 3:28 | 32.86 | Temp. of L. is now 30.695 C. |
| .... | ..... | Cooling of R. 0.16 degrees in 16 minutes. |
| .... | ..... | Cooling of L. 0.715 degrees in 65 minutes. |
| .... | ..... | Vol. of right hand 431 c.c. |
| .... | ..... | Vol. of left hand 407 c.c. |
| .... | ..... | Rectal temperature 37.05 C. |

also more persistent than when the anesthetic phalanges alone were exposed to the cold water.

After an interval of two months another examination was made.

December 19, 1912. Tested thermal sensibility of left hand before the experiment. No sensation to immersion in warm or cold water on middle finger, at least up to the upper third of the second phalanx. On the index finger no sensation on immersion at least up to a level well above the second interphalangeal joint. A perfectly safe level of immersion was thus determined beforehand. An hour before he had burned the flexor surface of the third phalanx of the index finger on a radiator without knowing it and there was a blister on it. He washed his hands in warm water before they were put into the bath at 2 p. m. Pulse 104 (sitting). Hands in calorimeter at 2:12. In testing, the anesthetic phalanges were passed through a hole in a thick plate of cork to avoid contact of sensitive skin with the vessel or water.

TABLE 3.—Experiment 3 with Right Hand in Calorimeter.

December 21, 1912. Right hand put into bath at 11:18 a. m. Left hand in air. Pulse 104 (sitting). At 11:28½ right hand put into calorimeter.

| Time. | R. | Notes. |
|---|---|---|
| 11:28 | 30.60 | |
| 11:29 | 30.53 | Room 22.7 C. |
| 11:30 | 30.54 | Room 22.9 C. |
| 11:31 | 30.57 | |
| 11:32 | 30.625 | Room 22.9 C. |
| 11:33 | 30.69 | |
| 11:34 | 30.75 | Room 22.9 C. |
| 11:35 | 30.795 | |
| 11:36 | 30.855 | |
| 11:37 | 30.91 | |
| 11:38 | 30.97 | Room 22.9 C. |
| 11:39 | 31.02 | |
| 11:40 | 31.07 | At 11:40 anesthetic phalanges put in water at 8.1 C. No sensation. |
| 11:41 | 31.095 | Room 22.9 C. |
| 11:42 | 31.12 | |
| 11:43 | 31.165 | Room 23 C. |
| 11:44 | 31.205 | |
| 11:45 | 31.25 | Room 22.8 C. |
| 11:46 | 31.305 | |
| 11:47 | 31.365 | Room 22.8 C. |
| 11:48 | 31.42 | |
| 11:49 | 31.49 | |
| 11:50 | 31.53 | At 11:50 anesthetic phalanges put in water at 42.8 C. |
| 11:51 | 31.55 | |
| 11:52 | 31.59 | Room 22.6 C. |
| 11:53 | 31.62 | |
| 11:54 | 31.675 | Room 22.6 C. |
| 11:55 | 31.73 | Room 22.5 C. |
| 11:56 | 31.77 | |
| 11:57 | 31.81 | Room 22.5 C. |
| 11:58 | 31.87 | |
| 11:59 | 31.915 | |
| 12:00 | 31.965 | At 12:00, 7 phalanges of left hand put in water at 42 C. |
| 12:01 | 31.995 | |
| 12:02 | 32.02 | Room 22.7 C. |
| 12:03 | 32.06 | Room 22.8 C. |
| 12:04 | 32.10 | |
| 12:05 | 32.155 | |
| 12:06 | 32.20 | |
| 12:07 | 32.26 | Room 23 C. |
| 12:08 | 32.25 | |
| 12:09 | 32.32 | Room 23.1 C. |
| 12:10 | 32.35 | |
| 12:11 | 32.39 | |
| 12:12 | 32.43 | Room 23.1 C. At 12:12 the 7 phalanges put in water at 8.2 C. |
| 12:13 | 32.46 | |
| 12:14 | 32.48 | |
| 12:15 | 32.51 | Room 23.2 C. |
| 12:16 | 32.535 | |
| 12:17 | 32.56 | Room 23.35 C. |
| 12:18 | 32.585 | |
| 12:19 | 32.605 | |
| 12:20 | 32.63 | Room 23.3 C. |
| 12:21 | 32.655 | At 12:21 whole left hand put in water at 43 C. |
| 12:22 | 32.68 | |
| 12:23 | 32.705 | Room 23.4 C. |
| 12:24 | 32.74 | |
| 12:25 | 32.78 | |
| 12:26 | 32.82 | |
| 12:27 | 32.86 | Room 23.3 C. |
| 12:28 | 32.90 | |
| 12:29 | 32.945 | Room 23.2 C. |
| 12:30 | 33.02 | Hand out of calorimeter at 12:30. |
| 12:44 | 32.86 | |
| ..... | ..... | Cooling of calorimeter R 0.16 degrees in 14 minutes. |
| ..... | ..... | Cooling of control calorimeter (without hand) from 30.53 to 29.76 degrees, or 0.77 degrees in 71 minutes. |
| ..... | ..... | Rectal temperature 37 C. |
| ..... | ..... | Vol. of right hand 431 c.c. |

In the experiment of December 19 the flow for ten minutes before the vasomotor reflex was tested was 10.63 grams per 100 c.c. per minute for the right hand and 10.23 grams for the left. For the first two minutes of immersion of the anesthetic phalanges of the left hand in cold water the flow in the right hand sank to 5.21 grams, rising again to 9.15 grams per 100 c.c. per minute for the remaining eight minutes during which the anesthetic phalanges continued in the cold water. On transferring the anesthetic phalanges to warm water the flow in the right hand diminished for the first four minutes to 7.80 grams per 100 c.c. per minute, to increase again to 9.45 grams for the remaining seven minutes of the period of immersion. When the whole left hand was immersed in the warm water the flow in the right hand for the first three minutes was 9.27 grams, which increased to 13.27 grams per 100 c.c. per minute for the remaining eight minutes of the period. The whole left hand was then put into cold water and the flow in the right sank to 7.90 grams per 100 c.c. per minute during the nine minutes of immersion, to rise again to 9.39 grams (over a period of eight minutes) when the left hand was dried and wrapped up.

TABLE 4.—SUMMARY OF BLOOD-

|  | Temp. C. of Calorimeter |  |  |  | Vol. of Hands, c.c. |  | Heat given off (small cals.) |  |  |
|---|---|---|---|---|---|---|---|---|---|
| Date. | Room. | R. | L. | Art. Blood. | R. | L. | R. | L. | In mins. |
| Oct. 15 | 22.6 | 31.32 | 31.35 | 36.65 | 447 | 436 | 2537 | 2273 | 16 |
|  | 22.8 | 31.61 | ..... | ..... | ... | ... | 155 | .... | 2 |
|  | 22.9 | 31.72 | ..... | ..... | ... | ... | 1053 | .... | 7 |
|  | 23.0 | 31.87 | ..... | ..... | ... | ... | 431 | .... | 3 |
|  | 23.1 | 32.04 | ..... | ..... | ... | ... | 1157 | .... | 5 |
|  | 32.2 | 32.23 | ..... | ..... | ... | ... | 638 | .... | 4 |
|  | 23.2 | 32.39 | ..... | ..... | ... | ... | 915 | .... | 4 |
|  | 23.3 | 32.55 | ..... | ..... | ... | ... | 956 | .... | 8 |
| Dec. 19 | 23.3 | 31.17 | 31.17 | 36.55 | 431 | 407 | 2219 | 2018 | 10 |
|  | 23.3 | 31.46 | ..... | ..... | ... | ... | 206 | .... | 2 |
|  | 23.3 | 31.64 | ..... | ..... | ... | ... | 1394 | .... | 8 |
|  | 23.3 | 31.87 | ..... | ..... | ... | ... | 567 | .... | 4 |
|  | 23.35 | 32.06 | ..... | ..... | ... | ... | 1153 | .... | 7 |
|  | 23.3 | 32.25 | ..... | ..... | ... | ... | 464 | .... | 3 |
|  | 23.3 | 32.50 | ..... | ..... | ... | ... | 1669 | .... | 8 |
|  | 23.2 | 32.81 | ..... | ..... | ... | ... | 1032 | .... | 9 |
|  | 23.25 | 33.01 | ..... | ..... | ... | ... | 1032 | .... | 8 |
| Dec. 21 | 22.9 | 30.82 | ..... | 36.50 | 431 | ... | 2012 | .... | 9 |
|  | 22.9 | 31.10 | ..... | ..... | ... | ... | 242 | .... | 2 |
|  | 22.9 | 31.33 | ..... | ..... | ... | ... | 1684 | .... | 8 |
|  | 22.6 | 31.58 | ..... | ..... | ... | ... | 416 | .... | 3 |
|  | 22.5 | 31.79 | ..... | ..... | ... | ... | 1448 | .... | 7 |
|  | 22.7 | 31.99 | ..... | ..... | ... | ... | 263 | .... | 2 |
|  | 22.95 | 32.23 | ..... | ..... | ... | ... | 1782 | .... | 10 |
|  | 23.25 | 32.54 | ..... | ..... | ... | ... | 1110 | .... | 9 |
|  | 23.35 | 32.70 | ..... | ..... | ... | ... | 440 | .... | 3 |
|  | 23.25 | 32.98 | ..... | ..... | ... | ... | 1160 | .... | 6 |

In both of the above experiments the two hands were originally in the calorimeters. In order to test the effect of the immersion on the anesthetic phalanges alone, the left hand had to be taken out and dried.

Although the drying was carefully done, it might be objected that the effect on the flow in the right hand following the first immersion of the anesthetic phalanges was really due to stimulation of the temperature nerves in the normal portions of the still moist left hand. Of course, this possibility could only apply to the first immersion of the anesthetic phalanges and not to succeeding immersions. Yet it was considered desirable to eliminate it and this was done in the experiment of Dec. 21, 1912, in which only the right hand was put into a calorimeter, the left being kept dry until the moment for immersion of the anesthetic phalanges. As in the previous experiment, a sheet of thick cork with a hole through which the anesthetic phalanges passed prevented any contact of the rest of the hand with the cold or warm water or the container.

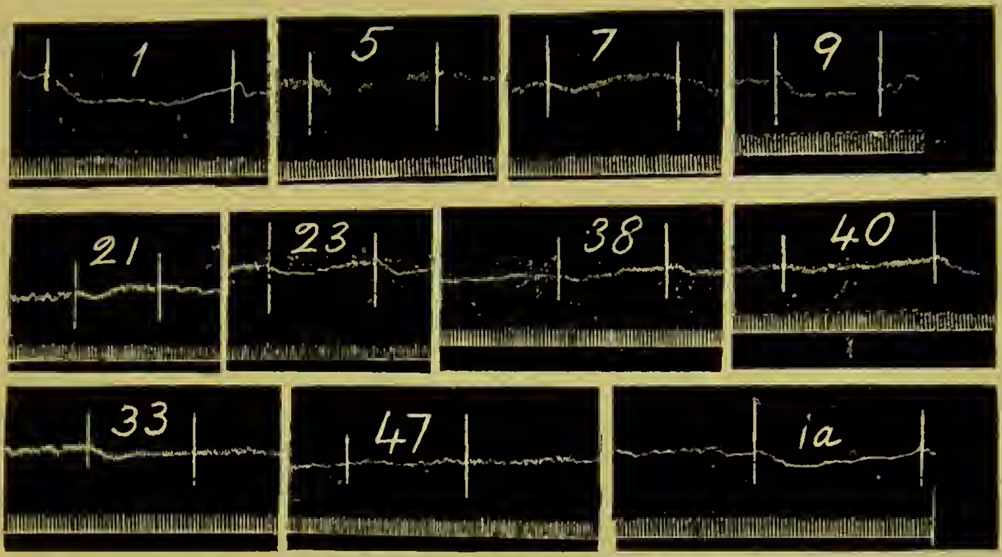
Flow Observations on O. C. W.

| Flow in Hands per min. | | Flow per 100 c.c. per min. | | |
|---|---|---|---|---|
| R. | L. | R. | L. | Notes. |
| 33.05 | 29.78 | 7.39 | 6.83 | Before vasomotor reflex tests. |
| 17.08 | ..... | 3.82 | ..... | Anesthetic phals. in cold water (first 2 mins.). |
| 33.90 | ..... | 7.58 | ..... | Anesthetic phals. in cold water (next 7 mins.). |
| 33.39 | ..... | 7.24 | ..... | Anesthetic phals. in warm water (first 3 mins.). |
| 55.77 | ..... | 12.47 | ..... | Anesthetic phals. in warm water (next 5 mins.). |
| 40.09 | ..... | 8.96 | ..... | Whole left hand in warm water (first 4 mins.). |
| 59.66 | ..... | 13.34 | ..... | Whole left hand in warm water (next 4 mins.). |
| 32.38 | ..... | 7.24 | ..... | Whole left hand in cold water |
| 45.82 | 41.67 | 10.63 | 10.23 | Before vasomotor reflex tested. |
| 22.48 | ..... | 5.21 | ..... | Anesthetic phals. in cold water (first 2 mins.). |
| 39.43 | ..... | 9.15 | ..... | Anesthetic phals. in cold water (next 8 mins.). |
| 33.65 | ..... | 7.80 | ..... | Anesthetic phals. in warm water (first 4 mins.). |
| 40.76 | ..... | 9.45 | ..... | Anesthetic phals in warm water (next 7 mins.). |
| 39.96 | ..... | 9.27 | ..... | Whole left hand in warm water (first 3 mins.). |
| 57.23 | ..... | 13.27 | ..... | Whole left hand in warm water (next 8 mins.). |
| 34.06 | ..... | 7.90 | ..... | Whole left hand in warm water (first 3 mins.). |
| 40.49 | ..... | 9.39 | ..... | Whole left hand dried and wrapped up. |
| 43.73 | ..... | 10.14 | ..... | Before vasomotor tests. |
| 24.89 | ..... | 5.77 | ..... | Anesthetic phals. in cold water (first 2 mins.). |
| 45.26 | ..... | 10.50 | ..... | Anesthetic phals. in cold water (next 8 mins.). |
| 31.31 | ..... | 7.28 | ..... | Anesthetic phals. in warm water (first 3 mins.). |
| 48.79 | ..... | 11.32 | ..... | Anesthetic phals. in warm water (next 7 mins.). |
| 32.39 | ..... | 7.51 | ..... | 7 phals. left hand in warm water (1st 2 mins.). |
| 46.37 | ..... | 10.75 | ..... | 7 phals. left hand in warm water (next 10 min.). |
| 34.60 | ..... | 8.02 | ..... | 7 phals. of left hand in cold water. |
| 43.27 | ..... | 10.04 | ..... | Whole left hand in cold water. |
| 61.02 | ..... | 14.15 | ..... | Whole left hand in warm water (next 6 mins.). |

It will be seen that the results are precisely the same as in the other experiments. For nine minutes before testing the vasomotor reflex the flow in the right hand comes out 10.14 grams per 100 c.c. per minute. For the first two minutes of immersion of the anesthetic phalanges of the left hand in cold water, the flow in the right sinks to 5.77 grams, to increase to 10.50 grams per 100 c.c. per minute for the remaining eight minutes of immersion. When the anesthetic phalanges were now immersed in warm water (the change from the cold to the warm water was made instantaneously without taking the phalanges out of the cork) the flow diminished for the first three minutes to 7.28 grams, to increase to 11.22 grams per 100 c.c. per minute for the remaining seven minutes of the period of immersion. Immersion in warm water of seven phalanges (second and third of index, middle and ring, and third of little finger) of the left hand, including the anesthetic phalanges, caused now a diminution of the flow in the right hand for the first two minutes to 7.51 grams per 100 c.c. per minute. For the remainder of the period during which these phalanges continued in the warm water (ten minutes) the flow rose to 10.75 grams per 100 c.c. per minute. For nine minutes during the immersion of these phalanges in cold water the flow in the right hand declined to 8.02 grams, a more persistent diminution than was caused by immersion of the anesthetic phalanges alone. When the whole left hand was subsequently put into warm water, an increase ensued in the flow in the right hand to 10.04 grams per 100 c.c. per minute for the first three minutes and 14.15 grams for the remaining six minutes of immersion, the greatest flow witnessed throughout the observation on this case.

In all three experiments, then, a change is produced in the blood-flow in the right hand when a portion of the left hand, totally insensitive to warmth or cold (as well as to other forms of sensation), is immersed in warm or cold water. Although the effect is smaller and more transient than that produced by immersion of the sensitive portions of the left hand, it is too definite to be attributed to accidental changes in the circulation.

The question has already been raised in discussing a similar reaction elicited from parts totally devoid of temperature sensibility in a pathological case,[1] whether afferent impulses capable of affecting vasomotor centers can be conducted by the nerves of warmth and cold sensation when they are unable to conduct impulses, at least in sufficient intensity, which give rise to temperature sensations, or whether the reactions are elicited through nerves, for example, those supplying the deeper structures, which can be excited by abrupt variations of temperature, although no result is produced in consciousness, and which can act as the afferent link in the vasomotor reflex arc. The possibility also presented itself that the blood in the anesthetic phalanges was sufficiently cooled or heated during the immersion to cause excitation of nerve endings in parts above the level of the anesthesia. In regard to this last possibility it must be remarked that since no sensation of heat or cold was experienced in such neighboring parts, the suggestion is really included in the ques-



Description of Figure 1.—Finger plethysmograph tracings. The tracings are to be read from left to right. The periods of immersion of the parts to which cold or warm water was applied are indicated by the vertical lines drawn through the curves. The signal trace which marked the moment of immersion and withdrawal has been omitted. Time is marked in seconds.

1. Right middle finger of S., a normal man, in plethysmograph. Left hand in cold water (8 C.) for the period comprised by the vertical lines drawn through the curve. The shrinking of the finger (fall of the curve) is evident.

5. Right middle finger of W. in plethysmograph. Whole left hand in cold water (8 C.).

7. Right middle finger of W. in plethysmograph. The anesthetic phalanges of left hand in cold water (8 C.).

9. Right middle finger of S in plethysmograph. Distal half of left hand in cold water (8 C.).

21. Right middle finger of W. in plethysmograph. Anesthetic phalanges of left hand in warm water (45 C.).

23. Right middle finger of W. in plethysmograph. Second and third phalanges of ring, and third phalanx of little finger of left hand in warm water (45 C.).

33. Right middle finger of W. in plethysmograph. The anesthetic phalanges of left hand in cold water (8 C.).

38. Right index finger of W. in plethysmograph. Anesthetic phalanges of left hand in warm water (43 C.).

40. Right index finger of W. in plethysmograph. Second and third phalanges of ring finger and third phalanx of little finger of left hand in warm water (43 C.).

47. Second and third phalanges of left index finger of W. in plethysmograph. Whole right hand in cold water (6.5 C.).

1-a. Right middle finger of W. in plethysmograph. The anesthetic phalanges of left hand in cold water (6.9 C.). This was the first immersion of the phalanges.

tion as already stated. In the hope of throwing some light on this question plethysmographic observations were made with a view of determining the latent period of the contralateral reflex elicited from the anesthetic phalanges. A finger plethysmograph was adjusted on the opposite hand. The moment of immersion of the anesthetic phalanges was written above the plethysmogram by an electromagnetic signal. It was foreseen that unless this latent period should turn out to be markedly lengthened no deductions could be drawn as to whether conduction of heat to or from a deeper layer than usual or convection of heat in the blood to neighboring parts was essential. For we neither know how rapidly heat would be conducted through a finger with the circulation going on, nor how great a change of temperature would be necessary to excite nerves which gave no sign by a sensation of temperature when the threshold had been reached. As a matter of fact, the latent period turned out to be quite small and not measurably greater than when the reaction was elicited from normal skin (Fig. 1). The description of the figure sufficiently elucidates the results. It is, however, worthy of special remark that when the second and third phalanges of the index finger of the left hand were enclosed in the plethysmograph no vasomotor reflex could be elicited from the right hand, even when the whole of that hand was immersed in warm or cold water. The meaning of this can only be that the efferent vasomotor fibers going to the index finger or their endings have not yet regenerated to the point at which they can conduct effective impulses. It is entirely in accordance with this that the spontaneous variations in the finger volume not connected with the respiratory movements and with a period covering several respirations which sometimes appear on the trace of the right middle finger, are absent from the trace of the left index finger. If these non-respiratory waves represent more or less rhythmical variations in vasomotor tone they must be absent from a part whose efferent vasomotor fibers are eliminated. We thus arrive at the conclusion that at a time when the efferent vasomotor fibers of a given part have not regenerated, afferent impulses from the same part elicited by heat or cold and producing no effect in consciousness can cause vasomotor reflexes. It must be remembered that the efferent vasomotor fibers in question are autonomic fibers whose cells of origin are in sympathetic ganglia, whereas the afferent fibers are cerebrospinal, their cells of origin being in the posterior root ganglia. In connection with the vasomotor paralysis of the anesthetic phalanges, the extremely rapid and persistent transudation of liquid into the blister on the terminal phalanx of the index finger is a matter of interest. The normal healing of the region, for it was not in the least delayed nor did any complication whatever appear, does not tally with the idea that any essential trophic influence had been removed by the injury to the cutaneous nerves.[3]

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[3] Additional note.—March 19, 1913. Peripheral reference of cold (to the tip of the index finger) has now been observed for the past ten days when a cold object touches a definite part of the scar in the forearm. It is elicited by the point (2 mm. in diameter) of a cylinder filled with ice but more intensely with a larger surface. The local response is inferior in intensity to the referred sensation. No peripheral reference of heat is obtained (confirmatory of Trotter and Davies[2]). No peripheral reference of cold as yet from any other part of the skin. The second and third phalanges of the middle and index fingers still give no response to hot or cold cylinders. But prolonged immersion in water at 43 C. (as in the blood-flow measurements) now causes the slow development of "something like a warm sensation," and in water at 8 C. "tingling as if from cold." Light stroking, contact with a blunt point, and pressure are now appreciated over the whole hand. The index finger is now hypersensitive to stroking (except the dorsum of the third phalanx) and appreciates pain on very strong pressure, as on biting the finger. Blood-flow observations in the hands show essentially the same features as at the previous examinations.

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# « SUR LA VITESSE DE LA CIRCULATION PULMONAIRE »

Par

**G.-N. STEWART**

### NOTE A PROPOS DU MÉMOIRE DE MM. LANGLOIS ET DESBOUIS

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Dans leur récent mémoire sur ce sujet [1], mémoire ayant pour base des recherches expérimentales faites sur le chien, Langlois et Desbouis écrivent : « Stewart prétend que sur le lapin, l'injection étant faite au moment de l'inspiration donne une lecture d'un temps plus court que si elle est poussée au moment de l'expiration, c'est-à-dire que l'inspiration active la circulation pulmonaire, l'expiration y mettant plutôt obstacle... Nous avons fait un certain nombre de recherches à ce sujet et ne sommes jamais arrivés à voir une différence sensible quel que soit le temps de la respiration auquel l'injection ait été poussée. »

En lisant mon mémoire, les auteurs ont probablement été trompés par le fait que pour éviter la répétition de la phrase « interruption de la respiration en position inspiratoire ou expiratoire » j'ai employé les abréviations « Insp » et « Exp » ainsi qu'il est soigneusement expliqué [2] « pour indiquer respectivement que l'observation était faite, les poumons étant distendus ou rétractés. Lorsque les abréviations « Insp » et « Exp » ne sont pas suivies d'un nombre mesurant le temps, l'intervalle entre l'arrêt de la respiration et l'injection de la solution saline était aussi court que possible, et ne dépassait pas en général plus de 4 secondes. — « Insp 20″ » signifie que l'injection n'était pas commencée tant que la respiration n'avait pas été arrêtée pendant 20 secondes dans la phase inspiratoire. « Exp for 20″ » a une signification correspondante. En aucun cas, la respiration ne recommençait avant que la lecture du temps de circulation n'ait été faite ».

Ce que j'ai établi concerne donc seulement l'influence de la position des poumons et du thorax quand la respiration est arrêtée dans le stade inspiratoire ou expiratoire pendant *toute la période* d'observation du temps de circulation.

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[1] *Journal de Physiologie et de Pathologie générale*, t. XIV, p. 282, 1912.
[2] Voir page 34.

Je n'ai pas fait d'observations sur l'influence que peut avoir le moment du cycle respiratoire auquel on pousse l'injection saline sur la vitesse de la circulation pulmonaire. Étant donné d'ailleurs le rythme respiratoire du lapin, dans les conditions expérimentales où je me trouvais, la durée de la petite circulation aurait été pratiquement contemporaine d'au moins un acte respiratoire complet, de telle sorte que si l'inspiration avait produit un effet quelconque, il aurait été masqué par l'effet contraire de l'expiration. Un essai de détermination du phénomène en question eût donc été sans objet.

Pour ce qui est de ma discussion relative aux causes de la différence des durées circulatoires pendant l'arrêt inspiratoire ou expiratoire, il n'est pas difficile de voir que la différence doit être plus prononcée, ou tout au moins plus facile à constater, dans le cas des petits animaux que dans celui des gros, chez les lapins que chez les chiens.

J'ai indiqué que dans un petit nombre d'expériences, si l'on faisait la moyenne d'un certain nombre de lectures, on ne pouvait voir aucune différence entre la durée de la circulation pulmonaire pendant « l'inspiration et l'expiration », c'est-à-dire pendant les arrêts inspiratoire et expiratoire, et j'ai montré que si mon explication est correcte, on pouvait s'attendre à quelques-uns de ces résultats (page 53). Le ralentissement de la durée de circulation pulmonaire dans le cas d'oxygénation imparfaite du sang, sur lequel j'insistais dans mon mémoire, est un facteur qui doit jouer un certain rôle dans l'effet de l'adrénaline, dans les cas où MM. Langlois et Desbouis observaient une apnée expiratoire prolongée, que le mécanisme de la variation de durée de la petite circulation dû à l'asphyxie et à l'adrénaline soit le même ou non dans tous les points essentiels.

Du point de vue technique, il n'est peut-être pas inutile d'insister une fois de plus sur le fait que la méthode qui consiste à noter le moment de l'injection et l'arrivée de la solution saline aux électrodes, au moyen d'un signal électro-magnétique sur un tambour, présente des avantages considérables sur l'emploi du chronomètre à arrêt quand les intervalles de temps sur lesquels doit porter la lecture sont petits. Des spécimens de tels tracés, pris par quelques-uns de mes étudiants aux travaux pratiques, et qui mettent très nettement en évidence l'allongement de durée de la circulation pulmonaire causé par l'excitation du vague, figurent dans mon *Manuel de Physiologie* (5ᵉ édition, p. 111, 1906; 6ᵉ édition, p. 126, 1910).

Pour beaucoup de recherches, je me suis servi pendant longtemps de la méthode téléphonique au lieu du galvanomètre pour mesurer des durées circulatoires. J'avais pensé dès mon premier travail à la méthode téléphonique, mais je fus détourné de l'employer par la crainte que les chocs d'induction de la force appropriée à la méthode de mesure, ne causassent des troubles sérieux en excitant les nerfs voisins. Mon dernier travail (*Journ. of Physiol.*, volume XXII, p. 159, 1897) montra que cette crainte n'était pas fondée, lorsque le vaisseau sanguin était bien isolé. Des électrodes recourbées en forme de crochet et faites de gros fils de platine supportent la portion dénudée du vaisseau.

Je n'ai jamais eu la moindre difficulté en travaillant avec des vaisseaux sanguins non ouverts, mais cependant, là où il n'y a pas d'objections à les ouvrir, et où l'on n'a pas à craindre la coagulation, des électrodes de platine introduites dans le courant circulatoire telles que les ont employées Langlois et Desbouis, et Steinhaus (*Archives internationales de Physiologie*, tome V, page 231, 1901), rendront possible l'emploi de courants plus faibles Dans quelques cas, la surface des électrodes pourrait être aussi grande que possible, ou elles pourraient être platinées si l'on voulait obtenir un minimum très exact. J'ai toujours pu arriver à obtenir une disparition suffisante du son quand la résistance du pont était compensée en prenant de larges électrodes de platines assez recourbées pour entourer la plus grande partie de la circonférence du vaisseau, sans être obligé de les platiner.

Le moment de l'arrivée de la solution saline est si facilement mis en évidence que depuis plusieurs années j'ai l'habitude de montrer l'expérience au cours en donnant aux étudiants, qui se les passent les uns aux autres, un groupe de cinq ou six téléphones tous reliés au pont par deux longs conducteurs. Pour les branches du pont de Wheatstone, il faut employer des résistances de 100 à 1 000 ohms, de telle sorte qu'une fraction suffisante du courant d'induction traverse le vaisseau sanguin.

Pour les petits animaux, la méthode au bleu de méthylène (Stewart, *Journ. of Physiology*, XXII, p. 160, 1897. *Manual of Physiology*, 1re édition, p. 157, 1896 ; 6e édition, p 181, 1906) est la plus simple et donne précisément les mêmes résultats que la méthode électrique, comme le montrent les observations simultanées dans lesquelles on injecte une solution saline fortement colorée par du bleu. La solution de bleu de méthylène est très facile à reconnaître dans un vaisseau sanguin isolé et non ouvert, grâce au brusque changement de couleur qu'elle produit, si le vaisseau n'est pas trop gros et bien éclairé. La carotide du lapin, du chat ou de petits chiens est parfaitement convenable pour ces expériences. Pour des vaisseaux comme ceux du mésentère, la lumière transmise peut être meilleure que la lumière réfléchie (voir Sollmann et aussi, *Journ. of Pharmacology and Experimental Therapeutics*, t. I, p. 435, 1909-1910). La décoloration du bleu est si rapide qu'une série de lectures successives peut être rapidement faite. Enfin, au moyen de l'ophtalmoscope, on peut même mesurer la durée de la circulation rétinienne par la méthode au bleu de méthylène (Stewart, *Proceed. of the American Physiolog. Soc.*, 27, 1895, et *Science*, t. III, p. 115, 1896).

L'utilité de l'une ou l'autre de ces méthodes pour l'étude de l'action des substances sur le calibre des vaisseaux ou sur les voies vaso-motrices dans différentes conditions, est considérable. Je me demande même si elles ont été suffisamment appréciées par les expérimentateurs qui ont fait des recherches dans cette voie, car, à ma connaissance, elles n'ont été employées que par Edmunds [1], par Sollmann [2] et par Steinhaus [3], puis enfin par Langlois et Desbouis.

Il y a déjà vingt ans [4] que des recherches faites sur la durée de la circulation dans la thyroïde du chien, avant et après la section du sympathique cervical et durant l'excitation de son bout céphalique, je concluais que le sympathique cervical contient des rameaux vaso-constricteurs pour le lobe thyroïdien correspondant. L'augmentation de durée de la circulation à travers la glande pendant l'excitation du sympathique cervical s'obtenait aussi bien lorsque le nerf était excité très bas dans le cou que juste au-dessous du ganglion cervical supérieur : ceci montrait donc que c'est au niveau de ce ganglion que les vaso-constricteurs thyroïdiens quittent la chaîne sympathique.

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[1] *American Journal of Physiology*, t. XVIII, p. 129, 1906.

[2] Sollmann, *loc. cit.*

[3] Steinhaus, *loc. cit.*

[4] Stewart, *Journal of Physiology*, vol. XV, p. 79, 1893.

La section du sympathique cervical provoquait une diminution nette de la durée de la circulation thyroïdienne, diminution qu'il fallait interpréter comme indiquant que les vaso-constricteurs de la glande avaient été sectionnés. Ces observations semblent être restées ignorées de François-Franck[1], qui n'en fait aucune mention dans son excellent travail sur l'innervation vaso-motrice de la thyroïde.

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[1] *Journal de Physiologie et de Pathologie générale*, t. X, p. 442, 1908.

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